BULLETIN No. 28. V. P. P.—82.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, Chief.

THE CULTURAL CHARACTERS

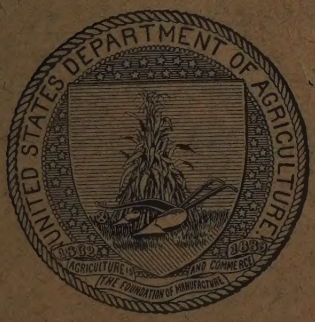
OF

PSEUDOMONAS HYACINTHI, Ps. CAMPESTRIS, Ps. PHASEOLI, AND Ps. STEWARTI—FOUR ONE-FLAGELLATE YELLOW BACTERIA PARASITIC ON PLANTS.

BY

ERWIN F. SMITH,
Pathologist, in Charge of Laboratory of Plant Pathology.

ISSUED AUGUST 6, 1901.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1901.

OFFICE OF PLANT INDUSTRY.

B. T. Galloway, *Director.*

AFFILIATED DIVISIONS.

Gardens and Grounds, B. T. Galloway, *Superintendent.*
Vegetable Physiology and Pathology, Albert F. Woods, *Chief.*
Agrostology, F. Lamson-Scribner, *Chief.*
Pomology, G. B. Brackett, *Chief.*
Section of Seed and Plant Introduction, Jared G. Smith, *Chief.*

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

SCIENTIFIC STAFF.

Albert F. Woods, *Chief of Division.*
Merton B. Waite, *Assistant Chief.*

ASSOCIATES.

Erwin F. Smith,
Newton B. Pierce,
Herbert J. Webber,
M. A. Carleton,
P. H. Dorsett,
Wm. A. Orton,
Ernst A. Bessey,
Flora W. Patterson,
Herman von Schrenk,[1]
Marcus L. Floyd,[2]
Thomas H. Kearney, Jr.

IN CHARGE OF LABORATORIES.

Albert F. Woods, *Plant Physiology.*
Erwin F. Smith, *Plant Pathology.*
Newton B. Pierce, *Pacific Coast.*
Herbert J. Webber, *Plant Breeding.*

---

[1] Special agent in charge of studies of forest-tree diseases, cooperating with the Division of Forestry, U. S. Department of Agriculture, and the Henry Shaw School of Botany, St. Louis, Mo.
[2] Detailed as tobacco expert, Division of Soils.

BULLETIN No. 28. V. P. P.—82.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, Chief.

THE CULTURAL CHARACTERS

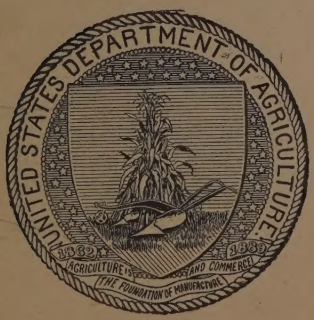
OF

PSEUDOMONAS HYACINTHI, Ps. CAMPESTRIS, Ps. PHASEOLI, AND Ps. STEWARTI—FOUR ONE-FLAGELLATE YELLOW BACTERIA PARASITIC ON PLANTS.

BY

ERWIN F. SMITH,
Pathologist, in Charge of Laboratory of Plant Pathology.

ISSUED AUGUST 6, 1901.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1901.

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., January 15, 1901.

SIR: I have the honor to transmit herewith the manuscript for a bulletin by Dr. Erwin F. Smith, of this Division, on the cultural characters of *Pseudomonas hyacinthi*, *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*—four one-flagellate yellow bacteria parasitic on plants. The first is the cause of a serious disease of hyacinths, described in Bulletin No. 26 of this Division; the second is the cause of a widely distributed and destructive disease of cabbages, known as brown rot and described from a practical standpoint in Farmers' Bulletin No. 68; the third is the cause of a serious disease of beans, and the fourth is believed to be the cause of a serious disease of sweet corn. The Bulletin also contains occasional references to *Bacillus amylovorus*, *B. coli* and other bacterial organisms which were used for comparison. It is the first exhaustive working over of an interesting group of plant parasites, concerning which practically nothing was known in 1896 when Dr. Smith began his studies. The work described is of a purely technical nature, but will be valuable to those in experiment stations and elsewhere who are engaged in investigating the bacterial diseases of plants. I respectfully recommend that the paper be published as Bulletin No. 28 of this Division.

Respectfully,

ALBERT F. WOODS,
Chief of Division.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	7
Growth in fluid media	9
Alkaline beef broth	9
Acid beef broth	11
Salted beef broth	12
Acid vs. Alkaline beef broth	14
Uschinsky's fluid	18
Milk and litmus milk	18
Growth on solid media	20
Loeffler's solidified blood serum	20
Nutrient gelatins	21
Nutrient agars	29
Potato	33
Coconut	35
Radish	36
White turnip	37
Yellow turnip	38
Rutabaga	40
Carrot	41
Sweet potato	43
Sugar beet	44
Sensitiveness to acids	46
Acid beef broths	46
Lactic acid	48
Potato broth	48
Malic acid	49
Cabbage juice	50
Tomato juices	51
Hyacinth broth	52
Feeble diastasic action	54
Iodine starch reaction	55
Growth on potato with addition of cane sugar	56
Growth on potato with addition of maltose and dextrine	56
Growth on potato with addition of diastase of malt	57
Potato starch in peptone water with diastase	57
Nutrient starch jelly No. 1	59
Nutrient starch jelly No. 2	63
Hyacinth starch jelly	64
Aerobism	65
Fermentation tubes	65
Growth in nitrogen	72
Growth in vacuo	76
Growth in hydrogen	79
Growth in carbon dioxide	83

Page.
Relative nutrient value of carbon compounds 85
Bouillon and peptone water with various sugars, etc 85
Crude vegetable substances 86
Sugar gelatin 87
Sugar agars 87
Sodium acetate 96
Nutrient starch jelly with sugars, gums, and alcohols 96
Temperature experiments 98
Thermal death point 98
Maximum temperature for growth 102
Optimum temperature for growth 108
Minimum temperature for growth 108
Formation of acids 109
Formation of alkalies 110
Rosolic acid test 110
Acid fuchsin test 113
Litmus 114
Reduction experiments 114
Methylene blue 114
Indigo carmine 115
Litmus 117
Tests for hydrogen sulphide 127
Formation of indol 128
Tests for nitrites 129
Peptonized beef broths 129
Peptonized Uschinsky's solution 129
Nitrate bouillon 129
Ferments 130
Cytase 130
Invertase 132
Diastase 133
Trypsin 134
Lab ferment 134
Oxidizing enzymes 134
Pigment studies 136
The yellow color 136
The brown pigment 140
Nature of the cell wall 143
Vitality 144
Length of life in culture media 144
Resistance to dry air 145
Resistance to sunlight 147
Resistance to heat 148
Resistance to acids 148
Resistance to alkali 148
Growth in presence of calcium sulphite 148
Growth over chloroform 148
Means of distinguishing the four species of Pseudomonas 149
Remarks on the yellow Pseudomonas group 152
Characters in common 152
Other species belonging to this group 153

ILLUSTRATION.

Page.

FIG. 1.—(a) Evolution of gas on adding hydrogen peroxide to potato culture of *Ps. phaseoli*.
(b) Uninoculated tube to which hydrogen peroxide has been added.. 135

The Cultural Characters of Pseudomonas hyacinthi, Ps. campestris, Ps. phaseoli, and Ps. stewarti, Four One-Flagellate Yellow Bacteria Parasitic on Plants.

INTRODUCTION.

The morphology and pathogenic properties of *Pseudomonas hyacinthi* were described by Wakker in 1883–1889,[1] and were redescribed by the writer in 1897 in Proceedings of the American Association for the Advancement of Science,[2] and in 1901 in Bulletin No. 26 of this Division.[3]

The morphology, cultural characters, and the pathogenic properties of *Pseudomonas campestris* were first established by Pammel (in part), in 1895[4] and were more fully described by the writer in 1897[5] and 1898.[6] In 1898 they were described also by Russell and Harding,[7]

[1] (1) Vorläufige Mittheilungen über Hyacinthenkrankheiten. Botanisches Centralblatt, Bd. XIV, 1883, pp. 315–316. (2) Het geel-of nieuwziek der Hyacinthen veroorzaakt door *Bacterium Hyacinthi* Wakker. Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1883. Haarlem, August, 1884. 8vo, pp. 4–13. (3) Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1884. Haarlem, May, 1885. 8vo, pp. 1–11. (4) Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1885. Haarlem, May, 1887. 8vo, pp. 1–5 and 27–37. (5) Contributions à la pathologie végétale: 1. La maladie du jaune ou maladie nouvelle des jacinthes, causée par le Bacterium Hyacinthi. Archives néerlandaises d. Sci. ex. et nat., Tome XXIII, 1889, pp. 1–25, pl. 1.

[2] Wakker's Hyacinth Bacterium. Proceedings of the Amer. Assoc. for the Adv. of Sci., 1897, p. 274.

[3] Wakker's Hyacinth Germ, *Pseudomonas hyacinthi* (Wakker). Washington, Government Printing Office, 1901, pp. 45, 1 pl., 6 text figs.

[4] Bacteriosis of Rutabaga (*Bacillus campestris n. sp.*). Bull. No. 27, Iowa Exp. Station, Ames, Iowa, 1895, pp. 130–135.

[5] (1) Science N. S., Vol. V, June, 1897, p. 963. Abstract of a paper read before the Biological Society of Washington, May, 1897. (2) *Pseudomonas campestris* (Pammel). The cause of a brown rot in cruciferous plants. Centralbl. f. Bakt. 2. Abt., Bd. III, July, August, and September, 1897, pp. 284, 408, 478, 1 pl.

[6] (1) The black rot of the cabbage. U. S. Dept. of Agr. Farmers' Bulletin No. 68, Jan. 8, 1898. (2) Additional notes on the bacterial brown rot of cabbages. Bot. Gaz., vol. 25, pp. 107–108. Amer. Nat. No. 32, p. 99. Both abstracts of a paper read before Soc. for Plant Morphology and Physiology in December, 1897.

[7] A bacterial rot of cabbage and allied plants. Wis. Exp. Station Bull. No. 65, Feb., 1898. (Issued in March.)

and by Russell.[1] More recently Harding has shown that the disease produced by this organism occurs in cabbage in various places in Europe;[2] and Hecke has demonstrated its occurrence in Kohlrabi in southern Austria.[2a]

Pseudomonas phaseoli was described briefly and named by the writer in 1897,[3] after securing numerous infections with pure cultures. The disease which it produces had been previously ascribed to bacteria by Beach,[4] and by Halsted,[5] as the result of a microscopic examination, but the organism itself had not been described, nor had it been shown by means of pure culture inoculations to what organism the bean disease was due. Quite recently the same or a very similar organism has been described briefly by Delacroix, who obtained it from diseased beans in fields near Paris.[6]

Pseudomonas stewarti was found in sweet corn and described in 1897 by Stewart,[7] who, however, established its pathogenic nature only inferentially. It was named with some additional characterization by the writer in 1898 from a culture furnished by Mr. Stewart for that purpose.[8] Doubt still remains as to its pathogenic properties, and must continue until the disease has been produced with pure culture inoculations from this particular species and under conditions precluding its origination by any other organism. Of the existence of a disease of maize due to bacteria no one who has examined specimens from Long Island or elsewhere can have a moment's doubt. The question as to what species causes it can be settled definitely only by successful pure culture inoculations.

The following pages were originally intended to form part of Bulletin 26 of this Division, but the manuscript grew to such an extent under my hands, and came to include so many references to related

[1] A bacterial disease of cabbage and allied plants. Proc. 11th, An. Conv. Amer. Col. and Exp. Stations, p. 86. (Issued in March, 1898.)

[2] Die schwarze Fäulnis des Kohls und verwandter Pflanzen, eine in Europa weit verbreitete bakterielle Pflanzenkrankheit. Centralbl. f. Bakt., 2 Abt., Bd. VI, 1900, No. 10, pp. 305–313.

[2a] Eine Bacteriosis des Kohlrabi. Zeits. f. das landw. Versuchswesen in Oesterreich, 1901, and subsequent letters to the writer. Inoculating from a pure culture furnished by Dr. Hecke, the writer has also recently produced the typical brown rot in cabbage.

[3] Description of *Bacillus phaseoli* n. sp. with some remarks on related species. Proc. Am. Assoc. for Adv. of Sci. for 1897, pp. 288–290.

[4] Blight of Lima Beans. N. Y. Ag. Exp. Station Bull. No. 48, new series, Dec., 1892, Geneva, N. Y., p. 331.

[5] A Bacterium of Phaseolus. Rept. of Bot. Dept. N. J. Exp. Station for 1892, pp. 283–285.

[6] (1) La graisse, maladie bactérienne des Haricots. Comptes Rendus, T. 129, p. 656. (2) Annales de l'Institut Agronomique, T. ——, p. ——.

[7] A bacterial disease of sweet corn. Bull. 130, Geneva Exp. Station, N. Y.; also 16th Ann. Rept. N. Y. Agr. Exp. Station for the year 1897, pp. 401–416.

[8] Notes on Stewart's sweet-corn germ, *Pseudomonas stewarti* n. sp. Proc. Am. Assoc. for Adv. of Sci. for 1898, pp. 422–426.

organisms, that, finally, it was decided to add still more references of this character and to publish it separately, making this bulletin, as far as possible, a monographic or comparative study of the cultural characters of the yellow species of Pseudomonas parasitic on plants. This statement will serve to explain the arrangement of the text. Under each subhead *Ps. hyacinthi* is the organism first considered, but whenever comparative studies have made it possible statements are added respecting the behavior of related species. Occasionally mention is made of species not closely related, e. g. *Bacillus amylovorus*, *B. coli*, *B. carotovorus*, and at the end I have noted some other species which belong to this group of bacteria, and which I have here designated THE YELLOW PSEUDOMONAS GROUP.

Some particulars have not been worked out as thoroughly as could be wished, e. g., (1) the relative nutrient value of nitrogen compounds, (2) the effect of antiseptics and germicides, but on the whole it seems best not to give any more time at present to these particular organisms, the main features of whose morphology and physiology have, it is believed, been made out correctly.

GROWTH IN FLUID MEDIA.

ALKALINE BEEF BROTH.

In test tubes of Weber's resistant glass, containing 10 c. c. of 1:2 alkaline beef broth[1] the fluid always showed a feeble clouding in 48 hours when inoculated with a 2 mm. loop from a fresh fluid culture of *Ps. hyacinthi* and kept at 23° C., or thereabouts. Also, when the tubes were inoculated with a much smaller number of germs, viz, as few as could be transferred from a fluid culture on the extreme tip of a platinum needle, the clouding always followed, being a little delayed

[1] This beef broth (stock 286b) was made as follows: Into a large beaker of Jena glass I put 1,100 grams of finely minced lean beef, covered it with 1,500 c. c. of distilled water (from a tin-lined copper tank), and set into the ice chest for 24 hours. The mixture was then strained as dry as possible through a clean towel which had been thoroughly washed in distilled water before using, an additional 800 c. c. of distilled water having been added previous to the straining. The result was 2,350 c. c. of red acid fluid. This was put into the steamer, warmed up to 100° C., and left at that temperature 45 minutes. It was then filtered through S. and S. paper, yielding when cold 2,000 c. c. of clear, pale, yellow fluid. This was then made up to 2,200 c. c. by adding distilled water. After thorough mixture of the broth and water by pouring, samples of the fluid were titrated against caustic soda, using phenolphthalein as indicator, 10 c. c. requiring 2.5 c. c. of $\frac{N}{10}$NaOH to exactly neutralize it. A fermentation tube filled at this time (25 c. c. of fluid) and afterwards inoculated with *Bacillus cloacæ* yielded 2 to 3 c. c. of gas, indicating the presence of muscle sugar. This acid fluid was designated 286a. To obtain stock 286b, 600 c. c. of this fluid was rendered exactly neutral to phenolphthalein by adding 7.5 c. c. of $\frac{2N}{1}$NaOH. On steaming one-half hour a slight precipitate came down. On filtering again the broth was perfectly clear and remained so. It gave a strong blue reaction with neutral litmus paper.

but in no way restrained. The importance of this fact will be apparent a little later when we come to discuss the effect of acid broths.

On the fourth day, in this alkaline beef broth, *Ps. hyacinthi* showed a small amount of yellow precipitate. On the 6th day there was less precipitate than in tubes of acid beef broth (stock 286a) 11 days old, but it was yellower. The clouding was so slight that a penholder was easily visible behind a thickness of two tubes.

On the eleventh or twelfth day there was more of the yellow precipitate than on the sixth, but it was not copious. Rolling clouds were visible on shaking, but no zoogloeæ. There was no pellicle, but now for the first time a feeble rim of germs was to be seen on the wall of the tube at the surface of the fluid. Under a Zeiss hand lens (× 6 aplanat) this rim appeared as a pale amorphous membrane thickly set with a series of roundish colony-like aggregates, which were white or yellowish, and which did not dissolve when shaken down into the fluid. Four days later the largest of these colony-like bodies were distinctly yellow, the smaller ones being white. On the twentieth day the fluid was uniformly clouded; there was no pellicle, and no ragged zoogloeæ were visible to the naked eye. The bright yellow precipitate on the bottom of the tube now covered a diameter of only 4 mm. The rim of germs was broad and filmy. It easily jarred off in large fragments, or as a whole, and fell to the bottom. It contained a great many zoogloeæ set at regular intervals in what still looked under a × 6 Zeiss aplanat like a homogeneous membrane. The upper, larger, and older aggregates were decidedly yellow, and set so closely as to form a yellow border on the upper rim of the ring, which was exposed to the air. The lower, smaller, and younger zoogloeæ on this ring were white, this part being submerged or barely out of the fluid. [Subsequent observations showed that these white zoogloeæ always became yellow with increasing age and size.] The greater part of the clouding was still attributable to individual germs, but some small zoogloeæ could now be seen in it, especially when examined with the hand lens. Under the compound microscope (Zeiss 16 mm. and 12 comp. oc.) the zoogloeæ on the rim looked like small, closely set colonies on an agar plate, i. e., they consisted of roundish, colony-like bodies on a paler, homogeneous looking membrane. Stained with gentian violet and examined under high powers the homogeneous substratum was seen to be composed of slender rods, which were often in short chains of 6 to 12 or more segments, the individuals forming the chains being distinct and of the same size and shape as those not joined.

On the thirty-third day there was a moderately abundant yellow precipitate, and the color approximated Ridgway's canary yellow. The fluid was less cloudy than it had been, but was still uniformly so. It was not turbid with zoogloeæ, but some small flecks were floating in it. There was no pellicle, but an easily detached, pale, fragile, homogeneous rim of germs, which was closely set with small, roundish, uni-

form-looking, colony-like aggregates. These did not dissolve readily in the fluid and all the larger ones were distinctly yellow and easily visible to the naked eye. The fluid had shown no acid reaction. It was now alkaline, and was not brown.[1]

On the fiftieth day the fluid was feebly and uniformly clouded, but much clearer than it had been. It was strongly alkaline to litmus; it was not ropy; there were no rolling clouds on shaking. There was no pellicle. The rim was 6 mm. wide and studded with zooglœæ; the largest of these were one-third mm. in diameter and yellow to the naked eye; the precipitate was still bright yellow and rather copious.

On the seventieth day the fluid was nearly clear, and there was no brown stain in it. It had evaporated from 10 c. c. to about 6.5 c. c. Eighteen days later the fluid was entirely clear.

On the one hundred and nineteenth day there was no brown stain, and large irregular crystals were present in the sediment.

Acid Beef Broth.

This broth was from the same stock as 286b, but no alkali was added. Its acidity was +25 of Fuller's scale, i. e., 25 c. c. of $\frac{N}{1}$NaOH would have been required to render 1,000 c. c. of this broth neutral to phenolphthalein. It was feebly acid to good neutral litmus paper. This fluid retarded growth slightly and was distinctly favorable to the formation of zooglœæ. The precipitate was more copious than in the alkaline beef broth and was duller yellow—a dirty Naples yellow. The clouding began in about 72 hours, when the inoculations were made with large loops from fresh fluid cultures and on the sixth day when the inoculations were made with as small a quantity of the fluid as could be lifted and seen on the tip of a platinum needle. Notes on one of eight cultures in this medium are given below:

Stock 286a, tube 11, February 4, 1898: Tube of resistant glass containing 10 c.c. of broth inoculated at 1 p. m. with *Ps. hyacinthi* from an alkaline beef broth culture (No. 1, January 29), which had been cloudy for three days and contained many actively motile germs. Only a tiny drop on the tip of a platinum needle was put into the tube, i. e., about 1.50 of a good-sized loop. February 5, clear; February 7, clear; February 8, clear. [Tubes exposed to the same temperatures as the alkaline beef broths.] Two check tubes of alkaline broth (286b) inoculated in the same way were cloudy on the third day. This broth exerts a distinct retarding influence which is especially noticeable when the dose of germs is small.

February 9. Clear.

February 10, 2.30 p. m. Very feebly clouded; some whitish flecks (zooglœæ) on the wall of the tube from top to bottom on one side.

February 19. Fluid turbid from numerous whitish flecks which are easily visible

[1] Throughout this bulletin "acid" and "alkaline" refer to litmus reactions unless it is otherwise stated.

to the naked eye. (No zoogloœæ visible in the two tubes of 286b held for comparison.) Rim well developed; most of its zoogloœæ are white, but a few are yellowish. A pellicle consisting of zoogloœæ held together by a film has gone to the bottom. Fluid homogeneous and now feebly alkaline to neutral litmus paper; in some of the tubes of this set the fluid has begun to clear a little at the top. Precipitate dirty yellow-white and rather abundant.

March 12. No new pellicle; fluid uniformly thin cloudy; no crystals. The zoogloœæ scattered through the fluid and lodged on the walls of the tube are very numerous, i. e., fifty times as many as in the two check tubes of alkaline beef broth. They consist of irregular, loose, rather large, whitish or very pale yellow-white flecks; rolling clouds are also visible on shaking. The tendency to form zoogloœæ is much stronger in this fluid than in 286b, but they also form in the latter after a time. There is a thin rim of germs on the wall of the tube for a distance of 3 mm. above the fluid; this rim bears several hundred small, roundish, colony-like zoogloœæ, most of which are now distinctly yellow—all the older larger ones. In the other seven tubes of this set most of this rim went down easily as a thin broken film on gentle shaking. The precipitate on the bottom of the tube covers a diameter of 10 mm. and is dull yellow—between wax yellow and Naples yellow. The color of the precipitate in the check tubes of stock 286b is brighter, and lies between gamboge yellow and chrome yellow.[1] The fluid is now plainly alkaline to neutral litmus.

April 13. Fluid nearly clear, no rolling clouds on shaking; no brown stain; moderately alkaline to neutral litmus paper. Precipitate more copious and certainly of a duller yellow than in the strongly alkaline broth. Rim of germs all of one kind, i. e., not contaminated, 6 mm. wide; all of the zoogloœæ on it are roundish, but only the older and larger ones are distinctly yellow.

April 25. The fluid has cleared, and there is no brown stain in it; when boiled, a vapor was given off which immediately blued moist neutral litmus paper.

Salted Beef Broth.

To determine the effect of sodium chloride upon *Ps. hyacinthi* the following experiment was instituted: Stock 529, which was an ordinary 1:2 acid beef broth (containing 1 per cent Witte's peptonum siccum and one-half of 1 per cent c. p. NaCl), was divided into two parts. To one was added an additional 1 per cent c. p. sodium chloride, forming stock 535; the other half was held as a check. The two culture fluids were then pipetted into clean test tubes of resistant glass and sterilized by steaming for a few minutes on each of three consecutive days. After some time the two sets of tubes, each of which contained exactly 10 c. c. of fluid, were inoculated at the same time and in the same way, i. e., with approximately equal numbers of bacteria from a well-clouded sugar bouillon culture (No. 6, October 29). Each of 6 tubes (3 of each sort) received a 2 mm. loop of the cloudy broth. The other 6 tubes each received as small a drop of the clouded fluid as could be seen distinctly on the end of a platinum needle. The experiment began at 3 p. m. November 5, 1899, and the subsequent observations were made at about the same time each afternoon. The following table, in which 0 denotes "clear," + "feebly

[1] Ridgway's Nomenclature of colors, 1st ed.

clouded," and + +, "very feebly clouded," shows the date at which clouding took place in these tubes, the temperature being the same, i. e., 18° to 22° C.:

TABLE I.—*Showing effect of sodium chloride on Pseudomonas hyacinthi in beef broth.*

Stock.	Method of inoculation.	Per cent of NaCl.	November—											No. of tube.
			6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	
529	Needle	0.5	0	0	0	#0	0	+							1
529	Needle	0.5	0	0	0	#0	++								2
529	Needle	0.5	0	0	0	#0	0	+							3
535	Needle	1.5	0	0	0	#0	0	0	0	0	0	0	++		4
535	Needle	1.5	0	0	0	#0	0	0	0	0	0	0	0	0	5
535	Needle	1.5	0	0	0	#0	0	0	0	0	0	0	0	++	6
529	2 mm. loop ...	0.5	0	#0	0	+									7
529	2 mm. loop ...	0.5	0	#0	0	+									8
529	2 mm. loop ...	0.5	0	#0	0	+									9
535	2 mm. loop ...	1.5	0	#0	0	0	0	0	0	++					10
535	2 mm. loop ...	1.5	0	#0	0	0	0	0	0	++					11
535	2 mm. loop ...	1.5	0	#0	0	0	0	0	0	++					12

There was a distinct slight retardation in stock 529, owing to the fact that no alkali was added to it other than that which was naturally in the peptone and perhaps, also, to the 0.5 NaCl which it contained. An examination of the table, however, shows that there was a very marked *additional* retardation in stock 535, which could be attributed only to the excess of sodium chloride. The retarding influence once overcome, growth proceeded, e. g., in tube 4 clouding was as twice as heavy on November 17 as on November 16, and in tubes 10, 11, and 12 it was twice as heavy on November 15 as on November 13. The date on which the clouding would have taken place in peptonized beef bouillon free from sodium chloride and neutralized to phenolphthalein by means of caustic soda is indicated in the table by # (see page 9).

Acid *vs.* Alkaline Beef Broth.

The following experiments were undertaken in 1899 to determine, approximately, the limits of growth of *Ps. hyacinthi* in acid and alkaline media. As a standard for comparison, I made use of a peptonized 1:2 beef broth neutralized to phenolphthalein by caustic soda, and well adapted to the growth of this organism. Portions of this stock were then acidified with varying quantities of malic acid, and others were rendered alkaline to phenolphthalein by an excess of caustic soda. Each test tube was of resistant glass, contained exactly 10 c. c. of the fluid to be tested, and was exposed to the same temperature. Except -80, all were inoculated June 11, from tube 8, May 14, a coconut culture, the growth of which had been delayed for some weeks in a U tube (nitrogen). Each tube received a very large number of germs and approximately the same number, i. e., a scant 2 mm. loop of the fresh yellow slime. Except −80, all of the cultures were carried through in duplicate. The approximate date of the clouding (temperature 25° to 30° C.) is shown in the following table, in which 0, is "clear," +, "feebly clouded," and ++, "very feebly clouded."

TABLE II.—*Showing growth of Ps. hyacinthi in peptonized alkaline, neutral, and acid beef broth, inoculated June 11.*

Acid and alkaline marked in degrees of Fuller's scale.[a]	June—					July—	
	12	13	15	19	29	23	26
−80	0	0	0	0	0	0	0
−40	0 0	0 0	0 0	0 0	0 0	0 0	0 0
−20	0 0	++ ?	+ +		Not one-fifth as much growth as in the following.		
0	0 0	+ + Several times as cloudy as the preceding.		Twice as cloudy as the preceding.			
+15 (Stock 473a, acid of beef broth).	++ 0	+ +	Clouding closely like preceding.	If anything, cloudier than the preceding.	A little cloudier than the preceding.		Rather more growth than in the preceding.
+30 (This and the following were acidified with malic acid.)	0 0	0 0	0 0	0 0	+ 0	Abundant bright yellow rim in each. Fluid now alkaline to litmus. Organism is able to make a moderate growth when it has overcome the malic acid. More growth in rim and pellicle than 0 or +15. Less precipitate than in 0 broth.	
+60	0 0	0 0	0 0	0 0	0 0	0 0	0 0
+90	0 0	0 0	0 0	0 0	0 0	0 0	0 0
+120	0 0	0 0	0 0	0 0	0 0	0 0	0 0

[a] Each degree on this scale corresponds to 1 c. c. of $\frac{N}{1}$ acid or alkali per liter of culture media, i. e., +15 means that the fluid is acid, and that 1 liter would require 15 c. c. $\frac{N}{1}$ alkali to render it neutral to phenolphthalein; −20 means that the fluid is alkaline, and would require per liter 20 c. c. $\frac{N}{1}$ acid to neutralize it.

From this table it would appear that the limits of growth for *Ps. hyacinthi*, under the conditions mentioned, lie between —20 and —40 on the alkaline side (probably near —35) and somewhat beyond +30 (probably near +40) on the acid side. For a long time I was in doubt as to whether any growth had taken place in +30, and it is not at all improbable that with the introduction of a lesser number of germs—e. g., a loop from a fluid culture—no growth would have taken place.

At the same time duplicate tests were made of a number of other bacteria and some of the results obtained are shown in the following table. Here, again, the tests were insufficient in number to bring out all of the peculiarities of the organisms. For instance, there should have been broths with intermediate grades of alkalinity and acidity, and for two of the organisms, *B. pyocyaneus pericarditidis* and *B. coli*, the series should have been extended on the alkaline side to at least —100. I have partially compensated for this by stating how soon the clouding appeared in certain of the fluids. In case of *Ps. stewarti* and *B. coli* the experiments should have been repeated in the +60 broth, since the growth was in any event feeble, and I was at times in doubt as to whether there had been any whatever. The —80 bouillon was inoculated June 13 with 2 mm. loops from fluid cultures 2 days old.

TABLE III.—*Showing growth of various organisms in alkaline, neutral, and acid beef broth (peptonized) with date of clouding.*

Organism.	Reaction of bouillon to phenolphthalein.								
	−80	−40	−20	0	+15	+30	+60	+90	+120
Ps. hyacinthi			2nd day.			18th day or later.			
Ps. campestris		2nd day to 5th day or later.				1st day or 2nd			
Ps. stewarti		1st day or beginning of 2nd				1st day.	?		
B. pyocyaneus pericard	5th day or 6th day.					1st day.			
B. amylovorus			1st day.			1st day.			
B. coli	Between 6th and 16th day.					1st day.	?		

21788—No. 28—01——2

Uschinsky's Fluid.

This fluid proved to be a very poor medium for the cultivation of *Ps. hyacinthi.* If only a few bacteria were put in, the fluid remained clear. If more were put in, growth appeared, but clouding was retarded (sometimes as long as 18 days) and was never other than feeble. On standing several weeks, there formed a feeble rim, at first white, then yellow, and a translucent pellicle dotted with roundish yellow zoogloeæ, which became yellower. If the rim or pellicle was shaken down into the bottom of the tube while it was still pale, it never acquired any deeper yellow. The fluid was never more than feebly clouded. The precipitate was bright yellow, but very scanty, amounting at the end of a month to a breadth of only 2.5 mm. on the bottom of the tube. At the end of 2 months seven-tenths of the original fluid remained. It had cleared, was free from any brown stain, and contained no crystals.

Very delicate white films and woolly flocculent bodies formed in this fluid and never became yellow. Under the microscope these colorless shreds and films consisted of enormous numbers of short, slender, motionless rods, so united that when the cover glass was jarred the mass moved as a whole. At first these bodies were supposed to be contaminations. The rods, however, were of the right size and shape for *Ps. hyacinthi*, and when these films and flecks were removed to beef broth, potato, or other suitable media only this one yellow organism developed. These bodies seemed so remarkable that a year later the experiments in Uschinsky's solution were repeated, with, however, identical results. *Ps. campestris* and *Ps. phaseoli* also grew feebly in this solution and with retardation, but without the films characteristic of *Ps. hyacinthi.* On the contrary, *Ps. stewarti* grew in it for a long time, and very copiously. *Ps. hyacinthi* grew very much better in Uschinsky's solution when 1 per cent Witte's peptone was added to it. In 3 weeks the growth in this peptonized fluid was 100 times as abundant as in the check tubes.

Milk and Litmus Milk.

The milk was obtained from a clean dairy and its reaction was amphoteric. It was used, nearly free from cream, in 10 c. c. portions, in test tubes of resistant glass. It was sterilized (about 24 hours after milking) by subjecting it, in wire crates, to streaming steam for 15 minutes at 100° C. on each of 4 consecutive days, and none of the many check tubes ever spoiled.

Many tubes of milk were inoculated with *Ps. hyacinthi* at different times. All gave the same result. For some time there is no visible change other than the formation of a yellow bacterial rim or pellicle, or both, with some yellow precipitate. Accompanying this growth

there is a slowly increasing alkalinity, which is first clearly visible on about the third to fifth day. Toward the close of the first week, or during the second week, a very slow separation of the casein (paracasein?) takes place. The first visible separation is usually apparent about the fifth to sixth or eighth to tenth day in the form of a millimeter-deep layer of clear whey on top of the milk, which is still entirely fluid. By the end of the third or fourth week the fine white casein has settled so that it occupies only about one-half of the fluid, the supernatant whey being pale yellow and transparent. Above this whey in old cultures there is always a 5 to 7 mm. wide, dense, bright-yellow bacterial rim on the tube. The casein does not set on the start and is never coarse flocculent. It finally becomes packed together in the bottom of the tube, but for a long time it consists of tiny separate particles which roll over each other easily when the tubes are shaken. This precipitated casein finally changes from white to yellowish and is slowly redissolved (peptonized). At no time during this precipitation and re-solution of the casein is there any acid reaction or any formation of gas. The whey from such cultures had a slightly bitter taste.

The reaction of the medium is best observed by adding to the milk enough litmus water to make it a deep lavender color; i. e., 10 c. c. of a saturated watery solution of c. p., blue, dry, lime-free litmus to 200 c. c. of milk. Many cultures were made in this medium with exact results. During the first 8 or 10 days the blue color very slowly deepens and the separation of the casein begins.[1] During the next 10 days or so the casein slowly settles and is still blue. Subsequently the litmus becomes more or less reduced, but at no time is there any appearance indicating the formation of any organic acid. When the litmus is not reduced the whey is pale wine red by transmitted light (normal color of the litmus), but is not red by reflected light. If after several weeks or months of growth such reduced or partly reduced cultures are killed by heating for 10 minutes at 56° C., and are then exposed to the air for some weeks, the color of the litmus returns. The undissolved casein is now distinctly blue and the whey is not red by reflected light.

Numerous tiny, white, centrally constricted, sheaf-like crystals of tyrosin appeared in old milk cultures. Crystalline plates presumed to be leucin also appeared.

Ps. campestris and *Ps. phaseoli* both act upon milk and litmus milk in much the same way. Neither produces any acid or gas. Both cause a slowly increasing alkalinity in the milk with the separation of the casein from the whey by means of a lab ferment. In both, a portion of this casein is redissolved (peptonized) with the formation of tyrosin and leucin. *Ps. stewarti*, on the contrary, does not precipitate the

[1] In one instance whey appeared in two tubes of blue litmus milk the fourth day. See table under Reduction.

casein or produce any other visible change in the milk, not even after several months, although it forms a distinct bacterial rim and a rather abundant bright yellow precipitate. (For action of this organism on litmus in milk, see Reduction.)

The kind of litmus used with milk sometimes has an important bearing on the results obtained, e. g., an acid reaction was obtained when *Ps. hyacinthi* was grown in milk colored with Sharp & Dohme's neutral solution of litmus, and, as this was the first litmus used, it might easily have led to erroneous conclusions had not further experiments been instituted with other brands of litmus. This litmus, which is very sensitive, keeps indefinitely, and is in use in many laboratories in this country, is preserved from deterioration (as I have since learned from the manufacturers) by the addition of 12 per cent ethyl alcohol. The acid which uniformly appeared in cultures of *Ps. hyacinthi* containing this litmus was not developed from the milk, but from traces of alcohol remaining in the medium after sterilization (see Formation of acids and Reduction—Litmus). This acid is volatile and smells like acetic acid. In some cases the acid which was produced from the alcohol inhibited the growth of the bacteria before the casein was precipitated, and this never did separate out (3 months).

A similar acid reaction was subsequently obtained by cultivating *Ps. hyacinthi* in blue litmus milk, to which drops of c. p. absolute alcohol had been added. In this case also only a slight amount of acid was formed, and it was not visible for some days. In both tubes the casein was thrown down by the lab ferment before there was any distinct acid reaction. Methyl alcohol was also tried in the same litmus milk, but no red reaction was obtained (56 days). The lavender-blue milk gradually became deep blue, and the whey separated slowly from the casein in the way already described. Evidently this organism can not break up wood alcohol.

For notes on the behavior in other fluid media, see Fermentation tubes, Sensitiveness to acids, Relative nutrient value of carbon compounds, Reduction of litmus, Formation of alkali, etc.

GROWTH ON SOLID MEDIA.

Loeffler's Solidified Blood Serum.

This medium was prepared from beef's blood in the pathological laboratory of Johns Hopkins Hospital under the supervision of Dr. Simon Flexner. It was solidified in test tubes in 15 or 20 c. c. portions, in long slants, with about 1 c. c. of fluid in the bottom of the V. The medium was in excellent condition for use, the surface being moist and the body of the substratum very light colored and clear. The slant surface of one of these tubes was streaked copiously, on June 5, from a coconut culture of *Ps. hyacinthi* 7 days old. Cultures of other yellow germs were laid on this medium at the same time, and

all were kept in the dark at room temperatures. During the first 5 days the temperature ranged from 23° to 31° C.; during the next 20 days the range was from 22° to 34° (25° to 30° most of the time), and during the last 6 days, from 29° to 37° C.

Result.—On the fifth day *Ps. hyacinthi* showed an abundant, smooth, wet-shining, bright-yellow growth the whole length of the slant, and a copious yellow precipitate in the fluid. There was no liquefaction, or, if any, only the merest trace on one side at the bottom of the slant. In corresponding tubes of *Ps. campestris* and *Ps. phaseoli* there was a distinct liquefaction the whole length of the streak. On the seventh day there was a slight liquefaction under the streak. This was plainest in the lowermost part, but was not one-twentieth as much as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. On the fifteenth day the precipitate continued to be brighter yellow, and there was decidedly less liquefaction than in corresponding tubes of the two organisms just mentioned. The serum of the long slant preserved its normal shape and color very well, even in the air, only the middle part and the sides just above the fluid being sunken in and dissolved away. On a scale of 10, the ability of these three organisms to liquefy this serum was marked 7, 5, and 2, *Ps. phaseoli* liquefying it most readily and *Ps. hyacinthi*, least readily. On the thirty-second day the precipitate in the V was 15 mm. wide and 7 mm. deep, and was still a trifle yellower than in corresponding tubes of *Ps. campestris* and *Ps. phaseoli*, but the liquefaction was decidedly less. The serum under the fluid still preserved much of its original color and was not liquefied, free access of air being apparently necessary, in case of each of these three organisms, to the operation of the chemical changes ending in liquefaction. About one-half as much fluid was now present in this tube as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. This fluid was strongly alkaline.

On the same medium *Ps. stewarti* made an excellent bright buff-yellow growth, but there was no trace of liquefaction (32 days).

Nutrient Gelatins.

Ps. hyacinthi grew better on beef broth gelatins made strongly alkaline to litmus (neutral to phenolphthalein) than on those which received less caustic soda and were feebly acid or feebly alkaline to litmus. It grew well, however, in beef broth gelatin first rendered neutral to phenolphthalein and then feebly acidulated with malic acid. A still better growth was obtained by adding cane sugar to this acid gelatin, the best growth of all being with +48 and +54 malic acid gelatin with the addition of 5 or 10 per cent cane sugar. The composition of the three gelatins on which the best growth was obtained is given below:

(1) *Stock 205.*—1,500 gr. finely minced lean beef; 3,000 c. c. distilled water. Mixed and put into a cool box for 24 hours. Then in steamer 1½ hours at 70° to 90°

C., and finally at 100° C.; squeezed fluid through a clean towel, and steamed again for 1 hour. Filtered through S. & S. paper, flasked, and sterilized forming stock 204. Fermentation tubes of this broth (25 c. c.) yielded about 1½ c. c. of gas with *Bacillus cloacæ*, showing the presence of muscle sugar. 1,700 c. c. of 204+255 gr. gelatin (Coignet Père et Cie gélatine extra). Gelatin soaked in broth 1½ hours, then heated in steamer to 100° C., cooled, added whites of 6 eggs (previously neutralized with c. p. HCl) to clarify, steamed, filtered, flasked, and steamed, added 17 gr. Witte's peptonum siccum, steamed and filtered; 1,500 c. c. of acid fluid remained; when titrated with caustic soda, 3.4 c. c. $\frac{N}{10}$ NaOH were required to exactly neutralize 10 c. c., using phenolphthalein as indicator; i. e., the acidity was +34. Reduced the acidity to +25 by adding 500 c. c. of distilled water. Then added 25 c. c. $\frac{2N}{1}$ soda, and steamed, filtered, flasked, and sterilized. The gelatin was steamed as short a time as possible on each occasion. This stock contained about 13 per cent of gelatin.

(2) *Malic acid gelatin.*—680 gr. finely minced, lean beef; 1,500 c. c. distilled water. Mixed and put into the ice box for 24 hours. Boiled three-fourths hour, filtered, cooled to 70° C., added 3 gr. NaCl, 12 gr. Witte's pept. sic., 100 gr. of brown German gelatin, and when the gelatin was dissolved the whites of 6 eggs, which were incorporated by repeated pourings. Steamed 40 minutes at 100° C., filtered through flannel, and titrated with caustic soda and phenolphthalein—acidity +45. Measured out 300 c. c. into each of three flasks and exactly neutralized each to phenolphthalein by addition of 6.7 c. c. $\frac{2N}{1}$ NaOH. To one flask was then added 7.8 c. c., to another 10.4 c. c., and to the third 11.7 c. c. of a malic acid water, 1 c. c. of which exactly balanced 13.75 c. c. $\frac{N}{10}$ NaOH; i. e., enough acid so that the gelatins should be approximately +36, +48, and +54 of Fuller's scale. These three acid gelatins were then filtered, tubed, and sterilized by steaming for a few minutes on each of three consecutive days. These stocks contained 8 per cent of gelatin.

(3) *Stock 244c.*—2,000 c. c. distilled water, 1,000 gr. lean minced beef, 300 gr. gelatin (one-half white French, one-half brown German), 20 gr. Witte's peptonum siccum. The beef was soaked in 1,200 c. c. distilled water for 1 hour, then brought slowly up to 65° C. on a water bath with constant stirring, then steamed 1 hour and filtered. The gelatin was soaked for a few hours in 1,000 c. c. of distilled water and then added to the hot beef broth, along with the peptone. Steamed 45 minutes, filtered, added 50 c. c. water to make up the 2,000 c. c. Fluid acid to litmus. Titrated with caustic soda, using phenolphthalein as an indicator. 2.85 c. c. $\frac{N}{10}$ NaOH was required to exactly neutralize 5 c. c. of gelatin, i. e., the acidity was +57. 14 c. c. of $\frac{2N}{1}$ NaOH was then added to 500 c. c. of the medium to make stock 244c, which was then flasked and sterilized. This stock, which contained 15 per cent of gelatin, was not perfectly solid at 24° C. A fermentation tube of the beef broth (25 c. c.) used for this gelatin yielded several c. c. of gas upon inoculating with *Bacillus cloacæ* indicating the presence of muscle sugar.

To test the effect of varying grades of alkalinity, stock 244c was compared with three other portions of the same gelatin to which different amounts of caustic soda were added, viz, (1) with a gelatin containing, per liter, 40c. c. less of $\frac{N}{1}$ NaOH (stock 244a); (2) with one containing, per liter, 20 c. c. less of the normal soda solution (244b); and, finally, (3) with one containing, per liter, 25 c. c. more of the normal soda solution (244d). These gelatins I commonly designate, fol-

lowing Mr. Fuller's scale[1] as +40 gelatin, +20 gelatin, 0 gelatin (neutral to phenolphthalein), and −25 gelatin. The litmus reaction of these gelatins was as follows: +40, feebly acid; +20, very feebly alkaline; 0, strongly alkaline; −25, very strongly alkaline.

As already stated, the best growth of *Ps. hyacinthi*, when peptonized beef broth was used, was in the 0 gelatin (stocks 205 and 244c), the next best was in the +20 gelatin. The difference in growth on these two gelatins was more striking at first than later on, the organism being able to partially overcome the inhibiting substances in the +20 gelatin. The growth in +40 and −25 gelatin fell far behind that in the other two. One was too acid and the other too alkaline.[2] These tubes were kept in a cool box at temperatures varying from 10° to 22° C. (most of the time 13° to 18° C.). In all the stab cultures the growth was best in the upper part, gradually fading out in the depths. The yellow color was also best developed near the surface, where there was freest contact with the air. There was no indication of yellow in the growth in the depths of the stabs.

The following notes represent the usual behavior of stab cultures in 15 per cent nutrient gelatin made neutral to phenolphthalein:

Stock 244c (10 c. c. of very clear gelatin in tubes 16 mm. in diameter): Stab made June 2, from a fluid culture 11 days old (cauliflower broth); one needle-thrust the whole length of the gelatin (5 or 6 cm.); tube kept in the cool box at temperatures varying from 10° to 22° C. (most of the time 13° to 18° C.). June 4 (range of temperature, 17° to 22° C.): A whitish thread distinctly visible one-half way down; it fades out gradually. June 5 (temperature, 13° to 15° C.): Stab whitish, feeble, visible three-fourths of the way down. June 8 (temperature, 13° to 16° C.): Stab begins to fade out two-thirds down; growth decidedly better than in the +20 gelatin—at least twice as much growth; slight liquefaction—i. e., a pit at the mouth of the stab, 3 mm. wide and 2 mm. deep. June 10 (temperature, 10° to 13° C.): Rather better growth than in the +20 gelatin, but fading out in the depths; no marked increase of liquefaction; surface growth pale yellow, rather dry looking, about 3 mm. in diameter; surface irregular. June 13 (temperature, 12° to 16° C.): A better growth than in the +20 gelatin, but fading out in the depths; surface growth about 4 mm. in diameter, pale yellow; pit of liquefaction 4 mm. wide and 2 mm. deep. June 18 (temperature, 14° to 18° C.): Growth a little better than in the +20 gelatin, but not now strikingly so; the stab fades out in the depths; surface growth pale yellow; pit of liquefaction only 5 or 6 mm. wide and 3 mm. deep; growth in the +40 gelatin is so slight as to be easily overlooked; growth in the −25 gelatin is confined to the surface and there is no liquefaction. June 28 (temperature, 13° to 19° C.): The pit of liquefaction is now 1 cm. deep and 1 cm. wide; it is a larger pit than in the +20 gelatin, and there is more growth in it, and also more in the depths of the stab, but the latter fades out at the bottom; the surface growth is distinctly yellow; in the +40 gelatin there is a barely visible growth in the upper part of the stab, a pit of liquefaction 3 to 4 mm. wide and 4 to 5 mm. deep, with a little whitish sediment at the bottom; in the −25 gelatin there is no visible stab, but a distinctly yellow surface growth 5 mm. in diameter, and a feeble liquefaction under it.

---

[1] Fuller: On the proper reaction of nutrient media for bacterial cultivation. Jour. Am. Public Health Association, Oct., 1895, p. 381.

[2] For the varying behavior of *Ps. campestris* in these four gelatins, see plate in The American Naturalist, March, 1899.

The growth in the streak cultures was better than in the stabs. Even on the best gelatin and when large loops of fresh fluid cultures were used the streaks came up rather slowly, i. e., in 3 to 5 days, at 18° to 24° C. When 2 weeks old, these streaks were usually 2 to 3 mm. wide and 4 to 5 cm. long. The growth was pale yellow, not very dense, finely granular under Zeiss ×6 aplanat, and often fine crenulate-serrate along the margins. The streaks were made with a medium-sized oese (2 mm. diameter), and the germs did not show much tendency to spread beyond the original streak.

In streak cultures on stock 205 a much better growth was obtained than with concomitant cultures of *Ps. campestris*, *Ps. phaseoli*, or *Bacillus tracheiphilus*. In 14 days, at 22° to 24° C , the streaks were about 2 mm. by 5 cm. with fine crenulate-serrate margins; the surface was very pale yellow and finely granular under Zeiss aplanat. There was no liquefaction at this date, but 6 weeks later all of the gelatin (10 c. c.) was fluid except a little in the bottom of the tubes and in the extreme top of the slant, and there was a moderate amount of pale yellow precipitate. During this period the tubes were in a cool box at approximately 20° to 24° C.

Some interesting results were obtained with the malic acid gelatins. They did not inhibit growth, as fluids of the same grade of acidity would have done, and in the +36 gelatin growth was not retarded. In the +48 and +54 gelatin growth was slightly retarded at first, and in the depths of the latter it was never as vigorous as in the less acid media, the separate colonies in the lower parts of the stabs always remaining smaller. After a time, however, the growth in the upper part of the stab and on the surface of the +48 and +54 gelatin outstripped that in the +36. On the fifth day there was most growth in the +36 gelatin and least in the +54. On the eighth day the retarding influence was still visible in the +54 gelatin. Subsequently it was overcome except in the depths. Two other interesting differences were observed. In 56 days, at 17° to 20° C., there was no liquefaction whatever in the +36 gelatin, and the color of the organism was a very pale yellow. In the +48 and +54 gelatins, on the contrary, the surface growth was bright yellow, and liquefaction set in at the end of the third week, involving the upper one-third of the 10 c. c. of gelatin in the course of the next 10 or 12 days. At the end of 56 days two-thirds to three-fourths of the gelatin in these tubes had liquefied.

A repetition of the tests with the +36 gelatin gave no different results. In 40 days, at 8° to 20° C. (mostly under 15°), there was no liquefaction and no bright yellow color, although the stab was well developed and the surface growth was 7 by 8 mm. in breadth. Additional cultures in the +48 and +54 gelatin yielded no new or different results. After 40 days there was an abundant bright-yellow growth, and the upper one-third of the gelatin was liquefied to the walls. The remain-

der of the 10 c. c. of gelatin was unchanged, the stab fading out in the depths to scattered round white colonies.

It would seem, therefore, that excess of malic aid favored liquefaction and the production of the yellow pigment. Possibly, however, these results are to be ascribed solely to changes in the physical character of the gelatin. The melting point was slightly reduced by the addition of the acid and was lowest in the stocks which received the most acid.

The most growth obtained on any gelatin was with +54 malic acid gelatin to which 10 per cent of cane sugar had been added. This was a slant culture kept in the ice chest at 10° to 25° C., for 6½ months, during all of which time it was overlooked, so that if there was any retardation of growth at first, there is no record of it. When examined at the end of 6½ months, there was a most copious growth, but no trace of liquefaction. At some time during the summer the ice was allowed to get low and the gelatin melted, allowing a copious bright yellow surface growth to fall to the bottom. Subsequently, with the addition of more ice, the gelatin resolidified and a new surface growth formed. When examined at the end of the 6½ months, there was a dense bright yellow rim 12 to 15 mm. wide, and a copious surface growth separated from the yellow precipitate, already mentioned, by a mass of solid gelatin free from browning and clear, except for tiny scattered masses of bacteria imprisoned when the gelatin resolidified. The bright yellow surface slime was still alive.

In the +48 gelatin with 5 per cent cane sugar, at the end of 40 days, at 8° to 20° C. (mostly under 15° C.), there was a compact bright yellow surface growth, 12 mm. in breadth and a distinct stab, but no liquefaction. This experiment was repeated at 12° to 20° C., using both +48 and +54 gelatin with 5 per cent cane sugar and continuing the experiment 30 days. During this time there was no liquefaction whatever in the +54 gelatin and only a very feeble liquefaction in the +48 gelatin, i. e., the distinctly yellow surface growth, 5 or 6 mm. in diameter, was sunken in slightly. In another experiment with +48 and +54 gelatin with 10 per cent cane sugar, kept at 7° to 21° C. (most of the time above 14° and below 19°), there was no liquefaction in 49 days.

The same results were obtained with 0 gelatin (stock 244c), to which 10 per cent cane sugar was added. After 61 days at 15° to 20° C. there was no liquefaction whatever in one tube and only the very slightest in the other, and no brown stain in either. The growth was better than in tubes of sugar-free gelatin which liquefied.

There can be no doubt, therefore, that, while powerfully stimulating growth, cane sugar in small doses retards and in large doses entirely inhibits the liquefaction of the gelatin, whether the medium is acid or alkaline.

In all of the gelatins liquefaction took place very slowly. In the 0 gelatins it seldom appeared earlier than the sixth day and often not until after the fourteenth day (temperatures 13° to 18° C., 18° to 21° C., and also 22° to 24° C.), and much later on gelatins not so well adapted to its growth. Even when once initiated the peptonization of the gelatins proceeded at such a snail's pace that 6 to 8 weeks usually elapsed before the whole 10 c. c. became fluid. This very slow liquefaction could not possibly have been due to the amount of gelatin used in my cultures, since this varied from 8 to 15 per cent, or to the fact that in preparing the media the gelatin was boiled only a very short time so as not to injure its solidifying properties. Neither does the temperature at which most of the experiments were made (8° to 21° C.) appear to have been the retarding cause, since liquefaction was not more rapid at higher summer temperatures. To test this, a tube of stock 244c was inoculated in June, 1897, and left for some weeks at room temperatures. These ranged from 25° to 34° C., the temperatures nearly all of the time being above 27° C., and often for many hours 29° to 32° C., i. e., near the optimum for this organism. The following notes on such a culture may not be without interest:

Stock 244c.—Tube of 10 c. c. inoculated June 14, noon, with a large loop from a beef-broth culture made June 3. Temperature, 25° C., gelatin fluid.

June 15, 1 p. m. (temperature, 28° C.). Feebly clouded throughout, no clouding down from the surface on shaking. Not so cloudy as concomitant cultures of *Ps. phaseoli.*

June 16 (temperature, 29° C.): Much less cloudy than a corresponding tube of *Ps. phaseoli.* Hundreds of tiny zoogloeæ have gathered into the top layers of the fluid gelatin and give to it, when shaken down, a granular appearance,

June 17 (temperature, 28° C.): Clear in comparison with a tube of *Ps. phaseoli.* Growth largely in shape of small zoogloeæ, some of which are again gathering into the top layers of the fluid gelatin.

June 18 (temperature, 26° C.): Very slowly increasing cloudiness with some tendency of zoogloeæ to gather into top layers of the fluid gelatin.

June 19, temperature 26° to 27° C.; June 20, temperature 29° C.

June 21 (temperature, 27° C.): A pale yellow rim on the tube at the surface of the gelatin. No pellicle, but some gathering of zoogloeæ and individual rods into the upper layers. Not much precipitate. On gentle shaking the fluid clouds down from the surface.

June 28 (temperature since last record, 25° to 31° C.): A moderate amount of yellow precipitate, much more than on the 21st. A copious yellow rim on the tube at the surface of the gelatin. Gelatin nearly clear until gently shaken, when it clouds down from the aggregation of germs in the surface layers. Numerous small zoogloeæ still visible. On putting into ice water the gelatin soon became solid.

July 8 (temperature since last record, 29° to 33° C.; i. e., very hot summer weather): Body of the gelatin nearly clear. A decided rim of yellow on the walls of the tube at the surface and some clouding of the upper layers of the gelatin. Considerable yellow precipitate. The fluid clouds down on shaking. It still solidifies quickly in ice water.

July 29 (temperature since last record, 26° to 34° C.): The gelatin has become clear. There is a yellow rim on the walls of the tube above the surface of the fluid and a copious yellow precipitate. The gelatin still solidifies in ice water, but it is only

semi-solid at 15° C., and is perfectly fluid at 18° C.; i. e., the melting point has been reduced 6 to 8 degrees, indicating a partial peptonization.

The most rapid liquefaction obtained was with a streak culture on stock 205. It was inoculated with a large loop from a fluid culture 10 days old and was kept at 18° to 24° C. On the twelfth day there was a thin, pale-yellow streak 2 to 3 mm. wide and 5 cm. long, in the middle part of which there was a small hole containing fluid gelatin. This liquefaction began the ninth or tenth day with a slight sinking in of this part of the streak. On the twentieth day the liquefaction involved about one-fourth of the gelatin (10 c. c.). On the twenty-ninth day fully three-fourths of the gelatin was fluid and there was a copious pale-yellow precipitate (temperature since last record, about 22° C.). Not until the thirty-ninth day was all of the gelatin fluid.

All of these gelatins contained some muscle sugar, which may have slightly retarded liquefaction, since various writers have shown for a number of bacteria that small doses of grape sugar retard and large ones prevent liquefaction.

In stock 208 (stock 205 + enough $\frac{2N}{1}$ c. p. HCl to make it neutral to sensitive neutral litmus paper) there was no growth whatever at 10° C. This inoculation was a streak the whole length of a long slant. It was made with a large loop of fluid from a beef-broth culture 7 days old and well stocked with living germs, as shown by the result of concomitant inoculations into other media. This culture was kept under observation 22 days. Inasmuch as the organism grew well in stock 205, and will also grow at 10° C., the failure of this tube was ascribed to the sodium chloride developed in the gelatin by the addition of the HCl, enough being produced to give a feeble taste of salt. (See p. 13.)

In the + 20 gelatin (stock 244b) this organism and *Ps. phaseoli* behaved much alike, both growing very much better than *Ps. campestris.*

In poured plates (+ 20 gelatin in Petri dishes) the buried colonies were round, roundish, or ellipsoidal, with smooth margins. No spindle-shaped colonies were seen and none or few having rough, irregular margins. (See plate cultures under Nutrient Agars.)

The size of these buried colonies in densely crowded plates (2,000 to 3,000 colonies per field of Zeiss 16 mm. aprochromatic and 12 compensating ocular), after 5 days at 13° to 16° C., was usually 16 by 16μ or 16 by 20μ. Some, however, were smaller, and others were as large as 24 by 24μ or even 28 by 32μ. The colonies were nearly colorless and very finely granular, with margins sharply defined and free from irregular outgrowths. Occasionally there were queer looking compound colonies resulting apparently from the growth of the component members of small zoogloeae. The plates were distinctly clouded to the

naked eye, but there was no liquefaction. Ten days later the colonies were decidedly larger, but otherwise much the same. The margins were still well defined and regular and there was no liquefaction. In less crowded plates of the same gelatin (200 to 600 colonies per field) at the end of 5 days (13° to 16° C.) the buried colonies were like those just described, only larger—28 by 28μ to 56 by 64μ, the greater number being 32 by 32μ to 36 by 36μ. Ten days later, at 12° to 16° C., the colonies had doubled in size, were round, roundish, or broadly elliptical, pale and finely granular (16 mm., 12 oc.), with clear, well-defined margins. The colonies in the deeper layers of the gelatin were decidedly smaller than those near the surface. The largest of the buried colonies, including some of the clumpy compound ones, were then a feeble tint of yellow. This color was only visible in the upper colonies. No spindle-shaped colonies were visible. Only two small pits of liquefaction were observed. These arose from surface colonies, of which very few were visible; i. e., the buried colonies did not easily break through and come to the surface, and free access of air appeared to be necessary for liquefaction.

None of the many pure cultures of *Ps. hyacinthi* in gelatin developed any gas bubbles (see Fermentation tubes), and the gas bubbles observed by Dr. Wakker in his gelatin cultures must be attributed to some contaminating organism.[1] Contrary, also, to Dr. Wakker's statements the gelatin did not become brown. In all of the gelatin cultures (tubes under observation from 3 to 6 weeks or more) it remained free from browning; i. e., was of the same color as when inoculated.

Ps. campestris and *Ps. phaseoli* both liquefy gelatin, and more readily than *Ps. hyacinthi*, but none of them are rapid liquefiers.

In nutrient gelatin stock 478, consisting of 1,000 c. c. stock 473b (beef broth acidity +17) and 100 grams of gelatin with 17 c. c. of $\frac{2N}{1}$ NaOH, *Ps. stewarti*, made a good growth. At the end of the forty-first day (temperature 17° to 22° C.) there was along the track of the needle puncture a thin line of growth, increasing toward the surface, and a dense, rather dry, and slightly roughened bright buff-yellow surface growth 7 mm. in diameter, but no liquefaction.

[1] The only gas that ever appeared in any of my cultures was in one of four gelatin tubes made June 23, 1897, in Dr. Wakker's manner, i. e., directly from the yellow interior of a disorganized bundle in an otherwise sound bulb scale. This tube was inoculated with great care to avoid external contaminations, and it appeared to be all right for some time, but after 22 days a gas bubble appeared in the gelatin near the bottom of the stab (temperature 12° to 18° C.). This was then the only evidence of anything wrong, but two weeks later the nature of the contamination became perfectly plain, the gelatin becoming fluid to the walls of the tube in the upper two-thirds, the upper part of the liquefied portion being greenish fluorescent and the bottom covered with a copious whitish precipitate with a little of the yellow *Ps. hyacinthi* mixed in. Undoubtedly this was an aerial contamination, as *Ps. hyacinthi* is never greenish fluorescent.

The following may be noted as some of the most characteristic peculiarities of *Ps. hyacinthi* on gelatin culture media:

(1) Liquefaction in neutral, acid, or alkaline gelatins, made with peptone and beef broth containing muscle sugar, proceeds very slowly at all temperatures (8° to 32° C.), reaching out to the walls of the tube long before it has involved the whole of the gelatin in stab cultures.

(2) The addition of 5 or 10 per cent of cane sugar greatly favors the long-continued growth of the parasite and does not interfere with the development of the yellow pigment, but entirely prevents liquefaction, or reduces it to an insignificant phenomenon easily overlooked.

(3) An extremely superficial, whitish, chemical film appeared after some weeks around the surface growth, even when cane sugar was added (see Nutrient Agars).

(4) None of the gelatins showed any browning or other stain of the substratum.

(5) No gas bubbles appeared, except in one tube which turned out to be contaminated.

(6) Quite unlike strong growing facultative anaërobic species, such as *Bacillus coli* or *B. cloacæ*, the stabs always faded out gradually in the depths, being best developed near the surface, and least in the deeper parts of the gelatin.

(7) The separate colonies, which in many instances formed the lower part of the stab, were always round or roundish, never spindle-shaped, and were never distinctly yellow, i. e., they were white or whitish, the free access of air appearing to be requisite for the development of the bright yellow pigment.

(8) Even in Petri dish cultures the surface colonies developed better than the buried ones, and the buried colonies in the surface layers grew better than those in the deeper parts of the gelatin.

(9) Peptonized beef broth gelatin which is only neutral or feebly alkaline to litmus exerts a retarding influence on growth. The reaction for best growth of this species lies somewhere between +15 and 0 of Fuller's scale. Litmus neutral gelatin also exerts a retarding influence on several other plant parasites, e. g., *Pseudomonas campestris* and *Bacillus amylovorus*.

NUTRIENT AGARS.

(1) Streaks of *Ps. hyacinthi* on brown agar No. 207 (+22) yielded a good pale yellow growth and the same sort of crystals as cultures of *Ps. campestris*, viz, large compound X-shaped crystals of magnesium ammonium phosphate.[1] These crystals were, however, less abundant than in cultures of *Ps. campestris* of the same age, and this was attributed to a feebler production of ammonia. The streak was still pale yel-

[1] The composition of this agar is given in *Centralblatt für Bakteriologie*, 2 Abt., Bd. III, p. 480.

low at the end of a month (living-room temperatures of March and April) and not at all sticky or gelatinous. Growth on this agar was retarded a little at first, but by the fifth day, when inoculated with large loops from beef-broth cultures a week old and kept at 21° to 23° C., there was a good, dense, yellow streak.

Fifteen months afterwards, a carefully preserved flask containing 500 c. c. of this same agar was opened and 100 c. c. of distilled water added to make up for what had slowly evaporated. The agar was then steamed, filtered, and filled into clean test tubes, forming stock 307. It was slightly browner and less elastic (more brittle) than when first made. Duplicate streak and stab cultures of this organism and also of *Ps. campestris* and *Ps. phaseoli*, both of which formerly grew well upon this agar, were made, using large loops of fluid cultures twelve days old. The loops were drawn lengthwise of the central part of the slant and were easily visible after the removal of the oese. *Ps. phaseoli* refused to grow on this agar, either in streaks or stabs (4 tubes, 56 days). *Ps. campestris* refused to grow in streak cultures and there was no visible growth in the stab cultures until after the sixth day (temperature 20° to 25°). This agar also exerted a powerful, restraining influence on *Ps. hyacinthi*. To the twelfth day the streak cultures showed no growth (temperature 20° to 25° C.). On the nineteenth day one of the streak cultures showed a distinct growth, but it was only 1 or 2 mm. by 10 mm. and was mostly in the agar. On the twenty-sixth day the streak measured only 25 mm. by 3 to 5 mm. The streak was dense, yellow, smooth, and wet-shining. The margins were thin and well defined. The organisms had grown down into the agar. On the fifty-sixth day the streak was 42 mm. by 5 to 8 mm. It was yellow, smooth, wet-shining, and contained several of the large X shaped crystals. No growth ever appeared in the other streak culture. Growth in the stab cultures was also much retarded and was very slow to appear upon the surface. This agar was not retitrated, to determine its acidity, but it was acid to neutral litmus paper, or, at least, not alkaline. When the moistened paper was dry, it seemed to be neutral.

(2) The following agar made by Mr. P. H. Dorsett was also tried:

1,000 c. c. of distilled water.
10 grams of Witte's peptonum siccum.
10 grams of agar.
2.5 grams of Liebig's extract of meat.

This fluid was cleared by the addition of the whites of 2 or 3 eggs and rendered moderately alkaline to litmus by the addition of carbonate of soda. It contained no muscle sugar and was +15.5 of Fuller's scale.

Repeated tests were made on this agar, usually in the form of streak cultures. There was no retardation of growth. The streak was distinct in 22 hours, at 27°, when the inoculation was made from a coconut

culture 8 days old, and in 28 hours, at 22° to 28°, when the inoculation was made from an agar culture 13 days old. This growth was thin, distinctly yellow, smooth, wet-shining, translucent, homogeneous-looking, and not scanty. There were no down-growths into the agar, and the margins, while thin, were well defined, i. e., not nebulous. Even on recently slanted agar the organism showed little tendency to spread widely. The streaks remained translucent for a long time, a penholder being easily visible through them after a month or more. No crystals were formed and there was no browning of the agar even in old cultures. (An undescribed, white, endospore-bearing Schizomycete, isolated from rotting tomato fruits, browned this agar readily.)

After a month or two the streaks began to dry out, but the surface remained smooth, even in old cultures, and was homogeneous looking, except that, after some weeks, colonies of the same species frequently formed on the surface of the yellow slime. Tested on the seventeenth, forty-seventh, and fifty-third days, with neutral litmus paper, the slime was feebly to plainly alkaline. On the sixty-sixth day it was strongly alkaline. No acid reaction was ever observed.

An extremely thin, whitish, chemical deposit appeared on the surface of the agar beyond the streak, after a week or two, and slowly increased, being best developed on the lower part of the slant where the growth was best. This film dissolved in 10 per cent acetic-acid water in about one minute.

On the forty-seventh day the slime consisted of short slender rods, single or in pairs. Four rods joined end to end were rare, and chains were very rare. After a long search only one chain was found (about 10 segments). In none of these tubes did the growth increase much after the second week, and it never became what might be called copious. No reticulate or shagreen surface ever appeared in any of these cultures. (See Sugar agars under Relative nutrient value of carbon compounds.)

Streak cultures of *Ps. campestris*, *Ps. phaseoli*, and occasionally of *Ps. stewarti*, were made for comparison. The behavior of these three parasites on this agar was much the same as that of *Ps. hyacinthi*. All grew without retardation, and after a few days there was about the same amount of smooth, translucent yellow slime. No crystals were formed in the agar and no brown stain appeared, even in old cultures. The whitish chemical film appeared around the streaks whichever organism was used, and in some cases it was noted that it was best developed in the lower part of the streak. In case of *Ps. campestris*, this film was examined microscopically and found to consist of very minute granular bodies, which were readily soluble in 10 per cent acetic-acid water, but did not show any decided crystalline structure when examined with the polariscope.

In one series of tubes, after five days on this medium, *Ps. hyacinthi*,

Ps. campestris, and *Ps. phaseoli* looked much alike, but the hyacinth germ was the brightest yellow and the cabbage germ the palest. On the seventeenth day *Ps. hyacinthi* was also noted as brighter yellow than the others. In another series of cultures the slime of *Ps. hyacinthi* was distinctly yellower on the sixteenth day than that of *Ps. campestris*, *Ps. phaseoli*, or *Ps. stewarti*. On the forty-seventh day the color of *Ps. hyacinthi* was saffron yellow (Ridgway, VI–4). The color of each of the other three organisms was paler, lying between buff yellow and chrome yellow. The cultures of these three organisms were also alkaline to litmus on various dates, and in cultures of the same age the slime on the seventeenth and forty-seventh day was more strongly alkaline than that of *Ps. hyacinthi*. All said, however, the cultures of all four of these organisms were much alike on this substratum at all stages of growth.

(3) Poured plates of *Ps. hyacinthi* were made in Petri dishes, using one of Mr. M. B. Waite's standard (litmus) neutral agars, in which *Bacillus amylovorus* had been found to make a good growth. In very crowded plates containing 8,000 to 10,000 colonies per field (Zeiss 16 mm. and 12 ocular), the agar, at room temperatures (25° C.), became milky cloudy on the fourth day. There were no distinct surface colonies, and the buried ones were irregular in outline, i. e., with ragged margins like the colonies of *Ps. campestris*. In a plate of the same age, but containing only about 600 buried colonies in each field (16 mm. 12 ocular), the colonies were larger, but otherwise of much the same character, i. e., roundish or somewhat irregular in shape with rough margins. No distinctly fusiform colonies were to be seen. Fusiform buried colonies were, however, observed in plate cultures made from Mr. Dorsett's agar.

In thin sowings of *Ps. phaseoli* on nutrient agar in Petri dishes (25 surface colonies and about 40 buried ones), on the seventh day (25° C.), the surface colonies were pale yellow, smooth, wet-shining, not piled up, and had thin, distinct margins. They were 1.5 to 4 mm. in diameter. The buried colonies were elliptical or bluntly pointed (0.6 to 0.7 by 0.3 to 0.4 mm.). The margin of the buried colonies was distinct but frequently a little roughened under the Zeiss aplanat. On the eleventh day some of the buried colonies were breaking through to the surface. The entirely buried ones were still small and elliptical, with either pointed or rounded ends. They were yellow in color and their margins were more or less roughened by small blunt projections. The surface colonies were now 3 to 8 mm. in diameter, smooth and wet-shining. Buried in the colony were a number of lighter and darker rings. The color was distinctly yellow, but pale rather than bright, i. e., somewhat like straw yellow. The margins were thin and well defined. Under high magnifications zooglœæ were visible in the colonies. There was nothing peculiar in

the marginal growth and the individual rods on the margin were not very distinct (Zeiss 16 mm. and 8 mm. apochromatics with compensating oculars up to No. 18).

POTATO.

More than 100 cultures of *Ps. hyacinthi* have been made on potato. This medium was usually prepared by steaming slant cylinders (5 to 6 cm. long by 1 to 1.3 cm. thick) in well plugged clean test tubes of resistant glass, in 1 to 3 c. c. of distilled water. Occasionally I made use of drier cylinders, only the curved bottom of the test tube being filled with water. The potatoes found in the Washington markets usually bear three steamings of 15 or 20 minutes each without cracking open or losing their smooth surface and white color, and, if they are prepared beforehand in a cleanly way, this short cooking on 3 consecutive days is sufficient to render them sterile.

The color of the organism on this substratum varies from bright yellow to pale or dirty yellow. Usually the color is distinctly brighter than in corresponding cultures of *Ps. campestris* or *Ps. phaseoli*. During the first week or two in most cases the color may be said to approximate Ridgway's Indian yellow (VI–5); i. e., it is nearly as bright as gamboge. As the culture becomes old the color dulls. In well-grown cultures not too old the color approximated Ridgway's wax yellow (VI–7). The color of the slime from a typical potato culture 30 days old was exactly Ridgway's gallstone yellow. Frequently the germs from very old cultures were brownish yellow in mass. The slime from a culture 48 days old was between ocher yellow and tawny olive.

Usually, at temperatures of 20° to 25° C., in inoculations made from broth cultures, the bacterial mass was not plainly visible along the streak until after 2 or 3 days. In one case it was distinctly visible in 24 hours, but then the temperature was 28° C., and the inoculation was with a mass of yellow slime from the surface of a potato culture. After a week or two the germ appeared in potato cultures as a thin, rather feeble, wet-shining, pale yellow or bright yellow growth, covering a part only or nearly the whole of the exposed potato, but showing no inclination to fill up the water.

There is, of course, a moderate clouding of the fluid around the cylinder, and after some days or weeks there is a scanty yellow precipitate which does not increase (14, 24, 41 days). All distinctly yellow growth is restricted to that part of the cylinder above the water. This growth is so thin that very often the slight irregularities of the surface of the substratum are not obscured and, as the fluid evaporates, the bacterial layer shows no tendency to follow down the sides of the cylinder and occupy the exposed surface of the potato. There is never any filling up of the fluid with yellow slime, such as always

appears in potato cultures of *Ps. campestris* and *Ps. phaseoli*. In comparison with either of these species the growth of *Ps. hyacinthi* on potato lags far behind, e. g., at the end of 2 days at 20° to 25° C. it is not one-twentieth as much, and at the end of 2 weeks it is not one one-hundredth as much. After the second week the hyacinth germ shows very little increase of growth on potato, whereas the other two germs continue to multiply for many days, converting the fluid in the bottom of the tubes into a solid mass of yellow slime even when as much as 2 or 3 c. c. of water is present (see Tafel VI, fig. 4, Centralb. f. Bakt., 2 Abt., Bd. III). This feeble growth on potato serves as a ready means of distinguishing this organism from the cabbage germ and the bean parasite, but not from some other yellow bacteria, e. g., *Ps. stewarti*.

The surface of this yellow growth, in *Ps. hyacinthi* even after several weeks, is usually homogeneous, smooth, and wet-shining. Very rarely, after the third week, I have seen a shagreen surface on the extreme upper part of the potato cylinder. After a few weeks (3 to 4) the bacterial layer is slightly sticky, often stringing up 1 to 2 centimeters when touched with the loop. After 3 or 4 weeks, when a considerable portion of the bacterial layer may be presumed to be dead, pale yellow, smooth, shining colonies, 1 to 3 mm. in diameter and gradually rounded up from the margin to a rather thick center, are sometimes seen dotting the surface. Zoogloeæ occur in this slime, at least after some weeks (30, 37 days), even when they are not visible in the form of shagreen. In one culture which was examined microscopically in water on the thirtieth day they consisted of numerous tiny ragged aggregates of 10 to 100 or more individual rods.

All the cooked potatoes I have ever tested have been feebly acid to litmus. This acidity is overcome by the growth of *Ps. hyacinthi*, the fluid first changing to feebly alkaline, and then becoming and remaining strongly alkaline (13, 22, 24, 30, 37, 56, 67 days).

The substratum out of the water is changed (as happens in case of many other bacteria) to a pale gray within a few days, and this color extends downward slowly into that part under the water, until after 3 or 4 weeks all is grayed; usually by the eighth or ninth day the gray color extended down under the water 1 centimeter. This color is a pale smoke gray, lighter than Ridgway's smoke gray (I–12). Its depth of color varies in different cultures, depending apparently on slight chemical differences in the potatoes used. The fluid in the bottom of these tubes remained free from color for a time, but after 3 or 4 weeks it became feebly browned. This brown color was distinct enough to be detected without check tubes, but it was never more than a weak stain (67 days).

The cylinders were firm and resistant between the fingers, even after the hyacinth organism had grown on them for 6 or 8 weeks, and their cellulose was certainly not acted upon to any marked degree. The

starch was also but little affected (see Feeble diastasic action). In young cultures there was no smell; in old cultures there was a feeble odor.

Ps. stewarti behaves on potato much like *Ps. hyacinthi.*

Potato cylinders on which *Ps. campestris* and *Ps. phaseoli* have grown are somewhat softened as if the middle lamella of the cell wall were attacked.

Coconut.

This substratum was made by putting clean, washed slices of coconut flesh into sterile, cotton-plugged test tubes, adding 1 or 2 c. c. of distilled water (from a tin-lined copper tank), so as to cover the lower one-third or one-half of the slice, and steaming 15 or 20 minutes on 3 consecutive days. The coconut flesh contains no starch and very little grape sugar (reducing substance), but is rich in oil. With the exception of rice it is the whitest culture medium known to the writer. All the yellow germs which I have tried make a satisfactory growth on this medium, and owing to its whiteness the contrast in color is very striking.

Ps. hyacinthi grows on this substratum without retardation. Cultures at room temperatures of 20° to 25° C. usually appeared in 36 to 48 hours, when not too sparingly inoculated, and made a good growth in 3 or 4 days. Growth continues for several weeks and usually becomes quite abundant (in one culture on the fiftieth day the bright yellow slime was over 1 mm. deep), but the organism shows no tendency to thicken the fluid or make it yellow, or to cover the submerged parts, any more than on potato, and there is little precipitate. The growth on this medium is smooth, wet-shining, and homogeneous. It is not sticky except in old cultures, which sometimes string up slightly. After 50 days in the ice chest the bacterial layer was not noticeably sticky, but it dissolved slowly in water and then lifted up 1 cm. when touched with the loop.

The color of *Ps. hyacinthi* on coconut is bright yellow. After 7 days' growth on coconut the organism was yellower than an equally good growth of the same age on turnip. At the end of the same period it was decidedly yellower than a corresponding culture of *Ps. campestris.* After 7 days and 25 days its color was about the same as that of a corresponding tube of *Ps. phaseoli.* After 49 days the color differed, if at all, from the color of a corresponding tube of *Ps. phaseoli* in being a trifle brighter, i. e., in containing less orange.

After 50 days at room temperatures of 18° to 27° C. its color was between Ridgway's lemon yellow and his gamboge yellow (VI–10 and 11). After the same period in the ice chest, at 7° to 15° C., its color was between canary yellow (light cadmium) and chrome yellow (Ridgway, VI–8).

No spores could be found in a culture which had grown in the ice chest for 50 days.

A culture at room temperatures was feebly alkaline to neutral litmus after 50 days. A culture which had been kept in the ice chest for the same length of time, and consequently was not so far advanced in growth, was distinctly alkaline, i. e., more so than the preceding. After 81 days in the ice chest a culture was strongly alkaline to neutral litmus.

No acid reaction was observed.

No brown pigment was developed. After 49 days at room temperatures the substratum was as white as when inoculated.

No cytohydrolytic action was observed. After the organism had grown on it for 81 days (ice chest) the substratum was as tough as when first inoculated.

No crystals were observed and there was no decided smell.

Ps. stewarti grew in much the same way on this substratum, but frequently made less growth. The color of its slime was buff yellow, and crystals were formed.

RADISH.

Slices of small, tender, red-skinned, turnip-rooted radishes were prepared in the same way as the potato.

On this substratum the hyacinth organism made a good growth, as the following record shows:

Stock 211.—Much water. Inoculated February 19, 5 p. m., from a beef-broth culture 14 days old, and kept at room temperatures.

February 22, 3 p. m. A good growth on the surface above the water, pale yellow, wet-shining. Fluid clouded, no precipitate.

February 26. A wet-shining, pale yellow growth over the whole exposed surface. A good growth, but not more copious than that in a corresponding tube of *Ps. campestris*. A moderate amount of precipitate. This is a yellower germ than *Ps. campestris*. It shows so, plainly, on all four media (radish, turnip, carrot, and coconut).

March 5. A copious growth. No brown pigment.

March 16. No brown stain.

April 9. The culture has begun to dry out, but there is still about one-half c. c. of fluid in the bottom of the tube. There is a thin pale-yellow precipitate. The substratum has changed color decidedly. The check tubes are still white, but the substratum in this one is of a color not easily described, i. e., unlike any in Ridgway's color system. It approaches his raw Sienna (V–2), and if that color had in it a very slight amount of brown it would closely resemble the color of this substratum. On long standing, therefore, a brownish pigment appears in tubes of radish.

A year later this experiment was repeated, using globose red and oblong white radishes. The results were substantially the same. There was a copious, very wet-shining, very pale-yellow growth, which never became bright yellow like that on coconut. In each case the substratum finally became brown, but this change took place very slowly, and the color never became deeper than a pale russet (64 days).

The slime was feebly alkaline on the thirty-fourth and sixty-fourth days whichever medium was used.

White Turnip.

Slices from the roots of smooth, green-leaved (nonglaucous), flat-bottomed, edible, white turnips were prepared in the same way as the potato cylinders.

The hyacinth organism grew well on this substratum and without any marked retardation. On the third day, at 21° to 23° C., the growth was very feeble in comparison with that of *Ps. campestris* or *Ps. phaseoli*. On the seventh day, at 20° to 23°, the growth was copious over the whole of the exposed part of the cylinder and the fluid was very cloudy, but there was little or no precipitate. On the twenty-second day growth was copious in the air and also in the upper part of the water, i. e., there was a better growth than in corresponding tubes of potato. After 54 days there was still a copious growth.

The surface of the slime was smooth and wet-shining, even in old cultures (54 days).

After 7 days at room temperatures the color in one tube was pale yellow, except the scanty precipitate, which was canary yellow. After 22 days the same culture was pale yellow. In another tube, on the seventh day, the color was unlike any in Ridgway's book, but approximated his Naples yellow (VI–18). This slime was plainly yellower than the equally copious growth in a corresponding tube of *Ps. campestris*. At the end of 25 days the slime in the upper part of the tube against the glass had developed a pale reddish-yellow color, quite in contrast with the color of a corresponding tube of *Ps. phaseoli*. There was also the merest trace of this color in the first cultures on radish. After 50 days at room temperatures the slime in one tube was "dirty yellow," while in another it was "pale yellow," i. e., much paler than in a culture of the same age on coconut. In mass, on white paper, this pale yellow slime was between Ridgway's buff yellow and maize yellow (VI–19 and 21).

After 54 days, at room temperatures, the slime showed no alkaline reaction, but was plainly acid to neutral litmus paper (only one tube tested). This red reaction was apparent at once and became stronger as the paper dried.

A brown stain slowly developed in the substratum, being clearly visible only after 2 or 3 weeks. On the twenty-second day and the thirty-eighth day the substratum was not browned as much as in corresponding cultures on yellow turnips. On the thirty-eighth day the color in one tube approximated Ridgway's russet. In another culture of the same age the brown was paler, approximating his tawny olive. On the forty-ninth day the substratum was darker than on the nineteenth, and was several shades darker than in a corresponding tube of

radish. At this time the color was approximately burnt umber (R. III–8), but it was a trifle lighter than that color and appeared to have a trace of red in it.

The stain in old cultures was always a distinct but feeble brown and differed from the stain of *Ps. campestris* principally in being a shade or two lighter.

Yellow Turnip.

This medium was made in the same way as the preceding. The turnips were of the same habit of growth as the white ones but were sweeter to the taste and were distinctly yellow. The relative amount of sugar in the two kinds was not determined.

Ps. hyacinthi grew remarkably well on this substratum and without any marked retardation. At room temperatures of 18° to 25° C. the bacterial layer was usually visible on the third day. A week after inoculation growth in the air was "copious" to "very copious," and growth in the water had been sufficient to produce a sirupy liquid. This growth continued for several weeks, entirely hiding the aerial part of the cylinder and converting all of the fluid (1 to 2 c. c.) into a solid slime which would not flow. In one tube, at the end of 8 days, there was 100 times as much growth as in corresponding cultures on potato. In other words, the organism behaved on this substratum exactly after the manner of *Ps. campestris* and *Ps. phaseoli* on potato.

At room temperatures the growth was smooth, wet-shining, and homogeneous-looking from the start, and it remained so for 2 months. There was never any shagreen surface or other surface indication of zooglœæ; nor was the dense copious slime sticky (eighth day).

The color of the slime was pale yellow; i. e., distinctly paler than on some other media. Examinations of 4 different cultures on the third, fifth, seventh, eighth, twelfth, twentieth, twenty-second, and thirty-second days all agree in this particular. On the fifth day the slime was a little brighter than Naples yellow. On the eighth day the color of the slime from another tube closely resembled Naples yellow, but was lighter yellow than the slime from a corresponding culture on carrot. In one instance the precipitate was canary yellow, while the aerial slime was paler yellow. On the fifty-fourth day, viewed without removal from the tube, the slime appeared to be russet color, but on putting a mass of it on white paper and comparing with Ridgway's plates its color was ochraceous.

On the eighth day the slime was distinctly alkaline to neutral litmus paper (one tube only was tested). In another tube, on the fifty-sixth day, the slime was feebly alkaline.

No stain of the fluid or of the substratum was visible during the first week of growth, but during the second or third week a brown color appeared and slowly increased in depth. On the twenty-second day

this color resembled tawny olive, but was paler. On the thirty-second day it was still only a pale brown. After 69 days the color of this pigment was between Ridgway's russet and burnt umber.

Bacillus amylovorus made only a moderate growth on this substratum, and produced no brown stain, but developed an acid.

After a year or two this test of *Ps. hyacinthi* was repeated at 20° to 25° C., using yellow globe turnips (a rough-leaved, nonglaucous sort). On the third day 5 sq. cm. of the slant surface was covered with a smooth, wet-shining slime, which was abundant enough to hide the substratum. On the seventh day there was a copious yellow, smooth, wet-shining growth over the whole cylinder and in the water, but no browning of the substratum. On the eighteenth day the fluid was so full of the yellow slime that it would not flow when turned bottom up, and there was a slight browning of the upper part of the substratum. On the twenty-seventh day there was a distinct pale-brown stain in the upper part of the substratum. On the thirty-fourth day the slime was neutral to neutral litmus paper. On the fiftieth day the color was between burnt umber and mummy brown, and the fluid was grown solid with the yellow-brown bacteria. On the sixty-fourth day the color of the substratum was burnt umber. The culture had a faint, peculiar smell. The outline of the substratum was preserved, but on being removed from the tube it was mushy soft to the fingers, and even to a piece of litmus paper which could be thrust into it. The substance was feebly alkaline throughout. There were some involution forms, but nothing resembling spores. Large crystals were present.

No starch remained, if any was originally present. The middle lamella was dissolved or greatly softened. The cell wall proper (of the turnip) was apparently intact, but for the most part the contents of the cells were gone, although some large and small rings of doubtful origin remained. With Russow's cellulose test many of these cells of the substratum did not stain at all, a few became deep blue, and a few deep purple. In most, the walls remained colorless, but the contents of the cells reacted pale blue. Corresponding results were obtained with chlor-iodide of zinc. The contents of the cells frequently became blue while the walls remained colorless or turned to brown or reddish brown. Doubt was thrown on these results, however, by the behavior of the check tubes, which also gave an uncertain cellulose reaction with these reagents; i. e., cell walls purplish in the chlor-iodide of zinc (on long soaking), and bright blue only in a few cells and parts of cells with Russow's test.

Ps. campestris also made a prompt and copious growth on this substratum, but there were some differences. On the seventh day the growth, while very abundant, was scarcely distinguishable in color from the substratum; i. e., it was plainly less yellow than that of *Ps. hyacinthi*. At this date the fluid was grown full of the bacteria

(solidified). On the eighteenth day the whole substratum was browned and this color was a much deeper brown than in the corresponding tube of *Ps. hyacinthi*. On the fiftieth day the color was burnt umber, and on the sixty-fourth day dark burnt umber. The slime was neutral or slightly alkaline on the thirty-fourth day, and feebly alkaline on the sixty-fourth day. The tissues were softened.

On this substratum *Ps. stewarti* made a thin buff yellow, slightly iridescent growth. On the seventh day there was one-fifth as much growth as in corresponding tubes of *Ps. hyacinthi* and one-tenth as much as in *Ps. campestris*. Growth did not increase much after the first or second week, and there was no browning or softening of the substratum. The culture was alkaline on the thirty-fourth and sixty-fourth days. After a time the water surrounding the turnip contained a moderate amount of buff yellow precipitate, but it never became thick or solid from excessive multiplication of the bacteria.

Rutabaga.

Test-tube cultures of this turnip (which had smooth glaucous leaves) were prepared with distilled water in the ordinary way (see Potato). The tests were made at the same time and in the same manner as on the yellow globe turnip, and the results were much the same.

With *Ps. hyacinthi* the growth was copious from the start, and not only covered the cylinder, but filled the fluid (solid). There was no stain of the substratum until after the twenty-seventh day, but this was covered by the bacterial growth so as not to be exposed anywhere directly to the air. On the fiftieth day the bacterial slime exhibited a smooth, wet, dirty, brownish yellow surface. The upper part of the substratum was now browned. The slime was acid to neutral litmus, leaving a distinct reddish color as it dried, and the cylinder was softened so that it mashed easily with a glass rod. The fluid was still plainly acid after adding 25 c. c. of water and stirring. On boiling only a trace of acid was given off in the steam. On continuing the boiling so that the fluid was reduced to 6 c. c. it was more strongly acid, and the acidity became still more pronounced on reducing it to 3 c. c. The boiled fluid had a slightly bitter taste. There was a slow evolution of gas and no white precipitate when this rather thick slime was put into barium chloride water (acid).

On this substratum *Ps. campestris* grew very promptly. By the seventh day the fluid was grown solid and the cylinder in the air bore on all parts a very copious, wet, shining, smooth, yellow growth. At this time there was already a slight stain of the substratum. This stain became more pronounced and extended to the whole substratum on or before the eighteenth day. This color (slime and substratum) gradually deepened through raw umber (fiftieth day) to mummy brown (sixty-fourth day). On the thirty-fourth and sixty-fourth days the

thick slime was acid to litmus, especially when diluted with distilled water. The tissues were softened and there was a peculiar smell, which was not rank or strong.

The behavior of *Ps. stewarti* on this substratum differed from that of *Ps. hyacinthi* and *Ps. campestris* in the same way as on the yellow globe turnip and was even more pronounced, so that it might be used as a means of distinguishing these organisms. The growth on the seventh day was about one-tenth as much as *Ps. hyacinthi* and one-twentieth or one-thirtieth as much as *Ps. campestris*.

On the eighteenth day the differences were as follows:

Ps. stewarti: Growth, buff yellow, thin, covering the whole of the air-exposed surface, but not dense enough to hide the slight irregularities of the substratum (not smooth). Surface slightly iridescent and with fine striæ (Zeiss × 6 aplanat), precipitate buff yellow and moderate in amount, water not grown full of the solid yellow slime, substratum not browned.

Ps. hyacinthi and *Ps. campestris:* Slime in the air copious, smooth, very wet-shining, pale yellow, surface not iridescent. Fluid grown full of the yellow slime (solidified). Substratum browned or ready to brown.

In old cultures of *Ps. stewarti* there was no increased growth, no brown stain, and no softening of the tissues. On the thirty-fourth day the thick slime would not wet litmus paper until water was added, when it gave an alkaline reaction. On the sixty-fourth day there was "a peculiar smell" and a feebly alkaline reaction. The iridescence persisted.

Carrot.

Cylinders of carrot were prepared in the same way as the potato cylinders.

Ps. hyacinthi grew well on this medium at 20° to 23° C., and generally without any distinct retardation. Usually growth was visible on the third day, and continued for several weeks, covering the aerial part of the cylinder with a bacterial layer a millimeter thick. The fluid in the bottom of the tube (1 to 2 c. c.) was also filled with a thick yellow slime, so that after 3 weeks it could usually be turned bottom up without flowing. Generally, though not always, growth was copious enough by the end of the first week to obscure the orange red of the substratum, which was not the case with *Ps. campestris*.

The surface was always wet-shining, even in very old cultures. In some it was smooth and homogeneous-looking from the start, and remained so. In others the surface was shagreened at first, but after eight days became smooth and homogeneous-looking. The bacterial slime was not sticky on the eighth day, in which particular it is very unlike *Bacillus tracheiphilus*. Subsequently (thirty-first and sixty-seventh days) it became slightly sticky.

The color on the fifth day was "bright yellow." On the eighth day it was between chrome yellow and maize yellow (R. VI–8 and 21). These two colors are compounded of varying amounts of orange cadmium, pale cadmium, and white. The slime in old cultures became darker, as if from admixture with a brown stain. On the thirty-first day the color was between ochraceous and raw sienna (R. V–2), being near the latter color. After sixty-nine days the slime in one tube was noted as "dark yellow" and in another as ochraceous to tawny ochraceous. In one of these tubes the carrot was observed to be decidedly deeper orange than when it was inoculated; i. e., than check tubes. On the fourteenth day the color and general appearance of this slime closely resembled that of a culture of *Ps. phaseoli* made for comparison.

On the eighth and twenty-third days the slime was distinctly alkaline to neutral litmus paper. On the same day the slime was more alkaline in a tube 31 days old than in one of the same age and origin, but in which the organism had grown for only 23 days; i. e., was restrained from growth by heat during the first week. In a culture 67 days old the slime was plainly alkaline.

After two and one-half months' growth, the carrot cylinders retained their form perfectly (2 tubes), but went into pulp easily under pressure of the fingers, as if the middle lamella had been partially dissolved. These cylinders had a soapy-feeling, and a feeble but distinct smell suggestive of ammonia and amin compounds.

In one instance crystals or crystal-like bodies were observed in the slime of old cultures (69 days).

Penicillium grew readily on carrot covered by this organism, was found associated with it in a number of the bulbs received from the Netherlands, and is mentioned by Dr. Wakker as sometimes occurring in badly affected bulbs.

A repetition of the carrot cultures in 1899 led to similar results.

In two test-tube cultures which were examined after seventy-two days the growth appeared to be typical for *Ps. hyacinthi*, but in one the carrot was browned and in the other not. In both cultures there was a feeble smell, like glue; in both the cylinders were softened and went to pieces under slight pressure of the fingers. In the one which was not browned the carrot was distinctly but feebly acid to neutral litmus paper (it was also acid on the forty-second day). In the other the surface slime was neutral to litmus (it was alkaline on the forty-second day). The interior of the carrot was also neutral or nearly so. Lead acetate-paper placed for six weeks in the mouth of this tube, below the cotton plug, was not browned. The cylinders are believed to have been derived from different carrots. Both cultures were inoculated from the same tube. The difference in brown staining is believed to be attributable to slight differences in the chemical composition of the carrots. (See Ps. phaseoli under The Brown Pigment.)

Sweet Potato.

This medium was prepared in the same way as the common potato. *Ps. hyacinthi* grew well upon it and with little, if any, retardation. Usually, by the end of the first week, at 18° to 25° C., the growth on the aerial part was copious. This growth did not stop early, as in case of the common potato, but continued for a long time, covering the whole of the exposed part and filling up the water with a solid yellow slime. In one set of cultures, at the end of the twenty-second day, the growth was 10 times as abundant as on the common potato, and on the fifty-sixth day 100 times as abundant. In another set of cultures, made some months later, there was "much more growth than takes place on the Irish potato." Usually, by the end of the third week, the 1 to 2 c. c. of water in the bottom of the tube was grown full of the yellow slime, so as not to flow when tilted.

The surface of this growth was wet-shining even in old cultures (55 days). At first the surface was smooth, but after some weeks it became uneven, i. e., thickly set with smooth-roundish prominences, which appearance I have designated as shagreen. This uneven surface remained wet-shining and homogeneous in color, and I have no doubt as to the purity of the culture. Even in cultures not older than one week the bacterial mass did not readily dissolve or shake apart in water.

The color of *Ps. hyacinthi* on this substratum at the end of the first week was wax-yellow to gamboge-yellow in one set of tubes and in another it was "bright-yellow." On the thirty-first day the slime was slightly sticky and its color in mass, on white paper, was maize-yellow. Examined microscopically at this time there were no spores but a great many slender chains (6 to 12 rods) mixed in with zoogloeæ and single and paired rods. After 55 days the slime in one set of tubes was "dull-yellow" and in another set "dirty yellow," but there was no distinct brown pigment. At this time, in one set of tubes, the slime consisted of a mixture of long and short rods, chains, and zoogloeæ. Some of the rods and chains were very long, extending one-sixth to one-fifth of the way across the field of the microscope (Zeiss 4 mm. apochromatic and 12 compensating ocular). At this time, in the other set of tubes, there were numerous roundish zoogloeæ embedded in the bacterial layer. These zoogloeæ were a little whiter than the body of the slime and dissolved slowly in water. Under the microscope they presented the same appearance as all the zoogloeæ of this organism, and I had no reason to suspect contamination.

An acid appears to be formed out of this substratum. After 31 days the slime from the bottom of a tube showed no alkaline reaction, but was neutral to good neutral litmus paper. After 56 days slime from the same cultures was still "neutral or slightly acid" to litmus,

there being no alkaline reaction whatever (2 tubes). After 55 days' growth the slime from another set of tubes showed no trace of alkaline reaction. That from one tube was "neutral or slightly acid" when stirred up in a drop of distilled water and tested with neutral litmus-paper, while that from another tube was "feebly acid." These results may be compared with those obtained from old cultures on common potato.

Sugar Beet.

The white sugar beet was prepared for use in the same way as the potato cylinders.

Ps. hyacinthi grew copiously on this medium and for a very long time. Usually, at 20° to 25° C., growth was visible by the end of the fourth day, and sooner if very copious inoculations were made; but in some instances growth did not appear until the sixth day, i. e., there was some retardation. Once, on rather dry cylinders 3 months old, the germ refused to grow, although it grew promptly in check tubes of freshly prepared coconut. Moreover, although inoculated very copiously on several different occasions, the organism could not be induced to grow in a flask containing several hundred grams of ground beets covered with 100 c. c. of distilled water. This failure was attributed to the acidity of the beet juice, since the organism grew readily in another flask, which was prepared at the same time and from the same beets, and differed from the preceding flask only in having the first 100 c. c. of water poured off after some hours and another 100 c. c. added.

Generally, by the end of the first week, the whole or nearly the whole of the aerial part of the cylinder was covered, but the early growth was not as copious as in corresponding tubes of *Ps. campestris*. In time this growth became very copious, and the fluid gradually filled up with a solid yellow slime. In 20 days (2 tubes) the growth was "much better" than on potato. In 22 days (another series) the growth was 3 times as much, and in 31 days 20 times as much as on potato. In 37 days (another series) there was a copious growth over the whole cylinder, and the fluid in the bottom (1 to 2 c. c.) was full of the yellow slime, there being at least 50 times as much development as on the potato. Judging from its appearance, this culture continued to grow for another month. After 55 days (another series) there was a much better growth than on potato. The growth in the air was copious, but not all of the fluid was filled with the slime. After 52 days (another series) the beet cylinder was entirely covered with a very copious growth and the fluid around the lower one-half was filled full of a yellow slime, exactly as if it were the cabbage or bean parasite growing on potato. In tubes of potato, inoculated at the same time from the same culture, the organism had

made only a feeble to moderate growth and had formed no yellow slime in the water, the contrast being very striking. After 135 days this culture on sugar beet was still fresh-looking, and the solid yellow slime where the water had been was 2 cm. deep.

The surface of this growth was always wet-shining, but sometimes it was smooth and at other times shagreened. Of two cultures examined on the fifth day, the one grown at room temperature was smooth, the one kept in the thermostat was shagreened. Of two other cultures examined on the twenty-second and thirtieth days, both grown at room temperatures, one was smooth and the other was shagreened, i. e., thickly set with smooth, roundish papillæ, which appeared gelatinous to the eye but lifted out readily when touched with the loop. The smooth culture was paler yellow than the other. Portions of the latter did not dissolve readily in water. Under the microscope this culture appeared to be all one thing. In a pale-yellow culture 30 days old there were no spores, but many dense aggregates (zooglœæ) not readily dissolving in water. The rods were short and slender, and no chains or motile elements were visible. In the same culture, after 55 days, there were colonies or zooglœæ in the surface slime. These had roundish margins and were paler yellow than the body of the slime. In a beaker of water they did not dissolve in one-half hour.

At first the cultures were not sticky (8 days), but eventually they became slightly stringy (30 days).

The color of the growth was distinctly yellow from the start, in most cases becoming bright yellow. In mass on white paper, on the eighth day, this color was between gamboge yellow and chrome yellow (Ridgway). After 21 days another culture was gamboge yellow and was several shades brighter than a corresponding culture on potato. After 17 days in the thermostat the color was "dull yellow." One culture remained pale yellow for 57 days. Several others grown at room temperatures were bright yellow after 2 months, and one was noted as still bright yellow at the end of 135 days.

Tubes inoculated from a culture 52 days old took readily, showing that a considerable portion of the culture was living.

An acid seems to be slowly developed in small quantities by the growth of the organism on this substratum. In one tube, at the end of 7 days, there was no acid reaction, the fluid being feebly alkaline to neutral litmus paper. On the eighth day, in a tube from another series, the slime was not alkaline and not acid, but exactly neutral. After 21 days, in a tube from another series, the fluid was feebly alkaline. On the thirtieth day, in cultures of another series, the yellow surface slime was not alkaline, but neutral or slightly acid. The fluid in the bottom of this tube was neutral, but the paper reddened on drying. The fluid, however, from a check tube was also neutral at first, but was equally and plainly acid when dry. After

55 days the yellow slime on the aerial part of the cylinder and the fluid in the bottom of this tube were both acid. There was no trace of any alkaline reaction, but this acidity was feeble, i. e., not much, if any, more pronounced than in the dried-out juice of the check tubes. On the thirtieth day, in another tube of the same series, a mass of germs from the top of the cylinder reacted feebly acid, or at least there was no alkaline reaction on neutral litmus paper. After 55 days a large loop of yellow slime from the same tube showed no alkaline reaction when rubbed on neutral litmus paper, not even when stirred up in a drop of distilled water. At the same time no acid reaction could be detected. The slime was neutral.

No brown stain appeared in any of these cultures (67 days).

See also Feeble diastasic action and Relative nutrient value of carbon compounds for additional notes on growth on solid media.

SENSITIVENESS TO ACIDS.

The failure of *Ps. hyacinthi* to produce any immediate symptoms, even when inserted into the hyacinth-leaf parenchyma by the million, the slow progress of the disease when it finally appeared, and the extent to which growth is restricted to the immediate vicinity of the vascular bundles, have been described in Bulletin No. 26. This behavior of the organism in the host plant, which resembles that of *Ps. campestris* in the turnip and cabbage, led me to suspect it might be very sensitive to acids. To test this supposition the following experiments were made:

Acid Beef Broths.

In all cases the rate of growth in beef broth made neutral to phenolphthalein was assumed as the standard.

(1) The first trials were with stocks 286a, 286b, and 286d. Stock 286a was a 1:2 beef broth, to which no sodium chloride or alkali was added, and the acidity of which was +25 of Fuller's scale. Stock 286b was a portion of the same broth rendered neutral to phenolphthalein (0 of Fuller's scale), by caustic soda. Stock 286d was a portion of 286a boiled down so that it was quite yellow and strongly acid, i. e., +80 of Fuller's scale. Each tube contained 10 c. c. of broth. All were inoculated at the same time from an alkaline beef-broth culture 4 days old, and were kept together in feeble light, at room temperatures of 20° to 24° C.

Result.—The *alkaline* broth (286b) clouded in 26 to 72 hours, according as the infection was made with a large loop or with a tiny drop from the tip of a platinum needle. Stock 286a (feebly acid) clouded in 48 to 168 hours, according to manner of infection (loop or needle). Stock 286d, whichever way inoculated, remained clear until the close of the experiment (49 days).

(2) Stock 300c was an old, partially evaporated flask of 286d brought back to its original volume by adding distilled water. Its acidity was +80; i. e., exactly 80 c. c. of $\frac{N}{1}$ NaOH would have been required to neutralize 1 liter, using phenolphthalein as the indicator. Stock 300b was a portion of 300c diluted with an equal bulk of distilled water, so that its acidity was reduced to +40. Stock 300a consisted of a portion of 300c diluted with twice its bulk of distilled water, the acidity being consequently reduced to about +27. Three tubes of each stock were inoculated from an alkaline beef-broth culture 11 days old. All of the tubes were kept together in feeble, diffused light, in well-plugged tubes of resistant glass, at room temperatures of 20° to 23° C. Two of each set were inoculated with large loops, the third with a tiny drop from the tip of a needle.

Result.—In 300c there was no growth whatever (21 days). In 300b growth was much retarded, the fluid remaining clear for 8 days, and probably for a much longer period. On the twenty-first day, when next examined, the two tubes inoculated by loop were feebly clouded, and showed a moderate amount of yellow precipitate. There were also quite a good many large yellowish flecks (zoogloeæ), on the walls and floating in the fluid. In the tube inoculated by needle the clouding was *very* feeble, there was only a *slight* precipitate, and there were no zoogloeæ. On the twenty-fifth day the fluid in the needle culture was neutral to sensitive neutral litmus paper, while in the loop cultures it had become feebly alkaline. In 300a clouding was visible on the sixth day in the loop cultures, and on the eighth day in the needle culture. Here also growth was retarded, but not so long as in 300b; e. g., on the twenty-first day the tube of 300a, which was inoculated by needle, was about twice as cloudy, and contained ten times as much precipitate as the tubes of 300b, which were inoculated by loop. The organism changed the fluids from acid to alkaline, and in the end (55 days) all of the cultures were much alike.

(3) The last experiment was repeated, more attention being paid to the time of first clouding in 300b. Each tube contained, as usual, 10 c. c. of broth, was tightly plugged, was inoculated with one loop (*oese* 2 mm. in diameter) from an alkaline beef broth culture 12 days old, and was set away in feeble light at room temperatures of 19° to 26° C. (mostly 20° to 21° C. during the first 6 days).

Result.—In 300a clouding was first visible on the sixth day, but was then very feeble. In 300b the fluid remained perfectly clear for 19 days. On the twenty-sixth day, when next examined, it was feebly clouded. In 300c there was never any growth (26 days).

Ps. campestris, *Ps. phaseoli*, and *Bacillus amylovorus* also refused to grow in 300c. On the contrary, *Ps. stewarti*, inoculated from a solid culture, grew in it for a long time and very luxuriantly, although clouding did not appear until the eighth day.

Lactic Acid.

Schering's diabetine (fructose) in 1 gram doses was added to test tubes containing 10 c. c. portions of standard nutrient agar (acidity + 15.5 of Fuller's scale), on which *Ps. hyacinthi* was known to grow well. This agar was resterilized, slanted, and inoculated by streaking, but no growth could be obtained (58 days). The inoculation was from an agar culture 13 days old, a large loop of the yellow slime of *Ps. hyacinthi* being rubbed thoroughly over the whole surface. That the culture used for inoculation was alive was shown by the fact that an inoculation therefrom into the same agar without the sugar produced a decided growth in 24 hours. This fructose agar was distinctly acid to neutral litmus paper, owing presumably to the presence of a small amount of lactic acid which is said by the manufacturers to be put into the sugar to improve its keeping qualities. Ten grams of this sugar required 10 c. c. of $\frac{N}{10}$ NaOH to render it moderately alkaline to litmus. When 0.7 c. c. and 1.0 c. c. portions of this alkaline sirup were added to tubes of this agar a substratum was obtained on which, after a time, the organism grew luxuriantly. The inoculations were made with a loop of slime from solid cultures. In the 0.7 c. c. tubes, growth was feeble during the first 4 or 5 days, then excellent and long-continued. In the 1.0 c. c. tubes, growth at the end of 7 days was still very feeble, i. e., not one one-hundredth as much as in the tubes containing only seven-tenths as much of the alkaline sugar. On the twelfth day there was about one-third as much growth; on the sixteenth day growth had much increased. After this the 2 sets of tubes looked much alike and the growth was at least 10 times as abundant as on the same agar without the sugar.

Potato Broth.

This broth was half strength, i. e., 1:4. It was made by putting 500 grams of thinly sliced potatoes into 1,000 c. c. of distilled water and heating on a water bath 2 hours at 40° to 55° C. The broth was then filtered, steamed one hour, cooled, made up to 2,000 c. c., filtered, tirated, and divided. Its acidity was +30 of Fuller's scale. For comparison, a portion of this broth received enough caustic soda to make it +24, another portion received one-third as much soda and registered an acidity of +28, a third portion received 1 per cent of Witte's peptonum siccum. This last was not titrated, but the peptone is known to give an alkaline reaction with litmus, and this addition must have considerably reduced the acidity of the fluid.

(1) Each tube contained 10 c. c. of broth and was well plugged. All were inoculated at the same time and were kept together in feeble diffused light, at room temperatures ranging from 20° to 25° C. Each tube was inoculated with a large loop from a well clouded alkaline beef broth culture 11 days old.

Result.—The simple potato broth (+30) powerfully retarded the growth of *Ps. hyacinthi*, the fluid remaining perfectly clear for 8 days, and probably much longer. On the twenty-fourth day, when next examined, the fluid was clouded, showed some precipitate and had become alkaline. The +24 and +28 broths were both feebly clouded in 72 hours. The peptone potato broth must have clouded somewhat earlier than the last two, as it showed distinctly more growth at the end of the third day. Query: What was the inhibiting substance represented by the difference between +30 and +28 and removed by the addition of this small amount of sodium hydrate? Could it be oxalic acid?

(2) The preceding experiment was repeated, all of the conditions remaining the same, except that fewer germs were put into the tubes. The inoculations were from an alkaline beef broth culture 12 days old, and each tube received a moderate sized loop instead of a large loop.

Result.—In the +30 broth, which was feebly acid to litmus, no growth ever appeared (31 days). In the +24 broth a very feeble clouding was visible in 68 hours. In the +28 broth clouding was visible in 44 hours. Feeble clouding also appeared within 44 hours in the peptone potato broth.

Ps. campestris and *Ps. phaseoli* also refused to grow in the +30 broth. *Ps. stewarti* grew in it readily.

Malic Acid.

This acid was added to gelatins (see Nutrient gelatins) and to the potato broth already described. A portion of this potato broth was measured out and enough of this substance was added to raise the acidity of the broth from +30 to +45. A tube was inoculated with a large loop of *Ps. hyacinthi* from the same culture used for the first potato-broth experiments. The tube was exposed to the same favorable conditions as the potato-broth tubes, but no growth ever appeared (55 days). A month later the experiment was repeated, inoculating with a moderate sized loop from the culture used for the second potato-broth experiments. This tube was subject to the same conditions as the latter, but no growth ever appeared (31 days). A month later, 2 more tubes were inoculated, using an enormous number of germs, viz, for each tube a mass of bright yellow slime 2 mm. in diameter, which was taken from the fresh surface of a starch-jelly culture 9 days old. These well-plugged tubes were kept in very feeble diffused light, at room temperatures of 23° to 30° C., but no growth ever appeared (80 days).

Ps. campestris and *Ps. phaseoli* also refused to grow in this broth. On the contrary, *Bacillus amylovorus*, inoculated from a colony, clouded it in 48 hours and in the end made a better growth in it than

in alkaline beef broth. *Ps. stewarti* grew in this broth without retardation. Three saprophytic bacteria, obtained by Mr. A. F. Woods from the surface of carnation leaves, also clouded this broth in 2 to 7 days, viz, a pink buff germ, a lemon yellow germ, and an orange colored germ, the latter probably identical with *Bacterium dianthi* Arthur and Bolley. (See also Growth in fluid media.)

Cabbage Juice.

This fluid was prepared by grinding green cabbage leaves and extracting the juice under pressure. No water was added. The leaves were from old, slow-growing, hothouse plants. This juice was divided into two portions, one of which was sterilized by forcing it through a Chamberland filter, and the other by steaming for a few minutes on 3 consecutive days. There was no difference in the acidity, each titrating +40 with caustic soda and phenolphthalein. The boiled juice smelled strongly of cabbage. Each stock was inoculated in the same way, i. e., with a small mass of bright yellow slime from a starch-jelly culture 28 days old. The tubes were well plugged and set in a dark place exposed to room temperatures of 22° to 33° C. (mostly 25° to 29°).

Result.—One tube of the filtered juice was under observation 44 days, but no growth appeared. Two tubes of the boiled juice were under observation, respectively, 29 and 44 days, but there was no growth. Five tubes of slant agar were inoculated at the same time from the same culture, and all took readily. Knowing that bacteria will tolerate more acid in a solid than in a fluid medium, 150 mgs. of Lautenschläger's neutral agar flour was added to one of the tubes on the twenty-ninth day. This was then steam sterilized, slanted, and the surface carefully streaked with at least a cubic millimeter of bright yellow slime from an agar culture 4 days old. This slant culture was under observation, in conditions favorable to growth, for 45 days, but no growth ensued, except on the wall of the tube above the slant in a place which was accidentally touched by the loop and where a little moisture condensed.

Ps. phaseoli and *Ps. campestris* also refused to grow in this acid cabbage juice; but when the fluid was solidified by adding 150 mgs. of the agar flour the latter made a very copious and prolonged growth—i. e., much better than on ordinary agar, although it was started upon it with great difficulty (3 copious inoculations). On the contrary, *Bacillus amylovorus* and *Ps. stewarti* grew in the boiled juice without retardation. The latter, inoculated from a solid culture, clouded the fluid (2 tubes) in less than 48 hours and made a very prolonged and copious growth. *B. amylovorus* grew nearly as well.

TOMATO JUICES.

Four tomato juices were tried, all from fruits of thrifty hot-house plants of one variety (Lorillard). The fruits were picked and sorted into groups as follows: (1) Stock 331, fruits red and ripe, with a fine odor, excellent for the table; (2) stock 332, fruits full grown and yellowish-green, i. e., commencing to ripen; (3) stock 333, fruits entirely green, but nearly or quite full grown; (4) stock 334, small green fruits, one-twentieth to one-fourth grown. The juices were obtained by pulping the fruits and extracting under pressure. These fluids were then filtered, steamed, filtered, filled into tubes, and sterilized by steaming 10 minutes on 2 consecutive days and 15 minutes on the fourth day. Each juice was carefully titrated for acidity and sugar content. Starch was abundant in the green fruits, but there was very little in the yellowish-green fruits and none whatever in the ripe fruits. Grape sugar was most abundant in the yellowish-green fruits. The acidity of the yellowish-green and of the ripe fruits was nearly the same, but undoubtedly they contained more than one acid, and the proportions were probably different. Each of these stocks was inoculated with at least one-half cubic millimeter of the yellow slime of *Ps. hyacinthi* from a coconut culture 7 days old, a check inoculation (which grew promptly) being made into alkaline beef broth. All of the tubes were kept together in feeble diffused light at room temperatures which ranged from 22° to 34° C. (mostly 25° to 28°) during the first 25 days, and after that 29° to 35° C., and occasionally for a few hours as high as 37° (Washington summer heat). The results obtained are given below:

(1) Stock 331. No growth (35 days). The acidity of the stock was +64, and the sugar content was such that 2.5 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4$ $5H_2O$ in Soxhlet's solution.

(2) Stock 332. No growth (35 days). The acidity of this stock was +68, and the sugar content was such that only 1.8 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4$ $5H_2O$.

(3) Stock 333. No growth (35 days). The acidity of this stock was +55, and the sugar content was such that 3.7 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4$ $5H_2O$.

(4) Stock 334. No growth (35 days). The acidity of this stock was +59, and the sugar content was such that 2.2 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4$ $5H_2O$.

The acidity here recorded marks the first perceptible trace of change of color on adding $\frac{N}{10}$NaOH drop by drop to 5 c. c. of the juice in 50 c. c. of water plus 1 c. c. of the standard alcoholic solution of phenolphthalein. More alkali was required to produce a bright pink, and, if this be taken as the standard color, then the readings would be, respectively, +74, +75, +65, and +72. Still more alkali was required to

produce a red which could not be made deeper. The readings when addition of more alkali did not deepen the color were, respectively, +111, +88, +81, and +93.

In each case these figures are the average of three titrations at room temperatures. Titrated boiling hot, each of the fluids required considerably *more* alkali.

Ps. campestris, *Ps. phaseoli*, *Bacillus amylovorus*, and *B. oleæ* also refused to grow in these juices. On the contrary, stocks 333 and 334 were well clouded by *Ps. stewarti* on the fifth day, and 331 became well clouded some time between the eighth and fifteenth day. In each of these 3 fluids this organism made a copious and prolonged growth, but it refused to grow in 332, although this contained more sugar than the other stocks; and even when reinoculated copiously from 331, after the latter had become well clouded, it remained clear. It is probable, therefore, that the limit of toleration of *Ps. stewarti* for the acids of the tomato lies between +64 and +68.

Hyacinth Broth.

Perhaps the most interesting result of all was obtained with hyacinth broth. This was made from 13 rather small bulbs of a single-flowered white variety of *Hyacinthus orientalis*. The bulbs had been kept in a closet in the laboratory all winter and had lost some water, but were not shriveled. In March the plateaus were removed (the most alkaline part of the bulbs) and the remainders were pulped and an effort made to extract the juice. Only a very sticky slime oozed through the bag, and this would not flow. I then added 100 c. c. of distilled water and squeezed out as much juice as possible under an iron press. An endeavor was made to pass the fluid through a Chamberland filter, but it would not go through with a pressure of 25 pounds per square inch. The fluid was then thoroughly steamed and filled into test tubes after filtering out a very copious white coagulum, consisting principally of nitrogenous substances, starch, and raphides. There resulted a hazy, yellow, acid fluid, which never precipitated entirely clear. Titrated with caustic soda and phenolphthalein, 1 c. c. exactly balanced 0.28 c. c. of $\frac{N}{10}$NaOH (first trace of color), and consequently the acidity was +28 of Fuller's scale. Pushed far enough to give a bright pink, the reading was +40. This fluid was moderately acid to neutral litmus paper. Four clean, well-plugged tubes, each containing 10 c. c. of this fluid, were inoculated with a large loop of *Ps. hyacinthi* from an alkaline beef-broth culture 2 days old, which broth was inoculated from a solid culture and had been cloudy for 24 hours. The tubes were kept in a dark place at room temperatures very suitable for growth, viz, 19° to 25° C.

Result.—This fluid exerted a profound restraining influence. In two of the tubes the first bacterial clouding appeared on the seventeenth day, at which time there was no rim of germs, pellicle, or precipitate. The other 2 tubes were not clouded on the seventeenth day, but on the thirty-seventh day, when next examined, there was a copious growth in each. A large loop taken from each of these 2 tubes on the eighth day and put into tubes of alkaline beef broth did not cloud the latter until the fifth day, from which we may infer that multiplication had gone on in the acid broth very slowly. When once the restraining influence was overcome, the organism ran riot in the fluid making a magnificent and long continued growth, more growth in fact than I had been able to obtain with any other fluid. On the thirty-seventh day the fluid in each tube was plainly alkaline to litmus; there was no pellicle but a dense bright yellow rim 4 mm. wide, and a yellow precipitate 5 to 6 mm. deep. The rim was homogeneous, i. e., not composed of scattered yellow zoogloeæ on a paler film, as was, however, the rim in tubes of alkaline beef broth inoculated directly from these cultures. This rim was wrinkled, or traversed crosswise, by many denser bands. The color of the bacteria was as bright as in the vessels of the host plant; compared with Ridgway's tables it exactly matched his chrome yellow (VI–8). On the fifty-second day all the tubes were alike. Each had a thick dark yellow ring above the fluid, and a copious, distinctly yellow pellicle. The fluid was nearly clear and distinctly pale brown, which was not the case with the broth in the uninoculated tubes. The yellow precipitate was three times as abundant as that obtained in alkaline beef broth, i. e., 6 to 7 mm. deep. The fluid was now strongly alkaline, and the germs were somewhat ropy. The cultures had a feeble, fishy odor suggestive of amin compounds. On boiling, gases were given off which immediately and strongly blued neutral litmus paper. Conducted into a tube of Nessler's solution, the vapor from the boiling fluid caused an immediate copious rusty precipitate. The same result was obtained by putting one-fourth c.c. of the filtered fluid into Nessler's solution, but no such reaction could be obtained from the uninoculated fluid. An attempt was made to determine the amount of alkalies present and the results are given, but I am not confident that either one is of any value. The fluid did not redden with a small quantity of phenolphthalein, but reacted with a larger quantity. Titrated in ice water with 6 c. c. of the standard solution of phenolphthalein, 3 c. c. of the fluid required 0.20 c. c. of $\frac{N}{10}$ HCl; titrated with neutral litmus, 3 c. c. required 0. 30 c. c. of $\frac{N}{10}$ HCl. It was difficult to drive off all the volatile alkalies by boiling, the blue reaction on wet litmus paper showing plainly in the steam when the fluid was half boiled away.

This experiment with hyacinth broth was repeated, inoculating a tube of the same stock with a moderate-sized loop from an alkaline beef-broth culture 12 days old, i. e., with more germs. All the other conditions were the same.

Result.—The first trace of growth was on the fifteenth day. On the nineteenth day there were distinct rolling clouds and a yellow rim, but no precipitate. Subsequently there was a copious growth and a very heavy precipitate. No crystals formed.

Ps. phaseoli and *Ps. campestris* both grew in this fluid, the latter much more readily than the hyacinth germ.

The discovery of this sensitiveness to acids furnished a satisfactory explanation of some perplexing contradictions obtained with unneutralized beef, potato, and cauliflower broths early in my study of *Ps. hyacinthi*. It also afforded a partial explanation of the slow progress of the disease in the host plant, but apparently not a full one, since once well established in the vessels, it is not clear why the parasite does not immediately advance into and destroy the acid parenchyma under cover of the alkalies which it produces. Evidently there are additional difficulties to be overcome, one of which will be discussed in the following section.

FEEBLE DIASTASIC ACTION.

The meager development on cooked potato led to the belief that something in this substratum inhibited the growth of the hyacinth organism. In the beginning it was thought that the feeble growth might be confined to certain varieties of potatoes and that on others a better growth could be obtained. To test this, cultures were made on a variety of tubers, new and old, but with the same result. Subsequently tubers were procured from a variety of soils and from climates as different as New York and Florida, but there was little difference in the amount of growth. The growth was comparatively feeble no matter what the age or origin of the potato. It was then thought that possibly the acidity of the potato might be the restraining cause, and dilute caustic soda was added to potato cylinders, so as to render them neutral or feebly alkaline after they were steamed. On such cylinders the organism grew little if any better than on the untreated potatoes (41 days), and this hypothesis was also abandoned. I then began to suspect that the feeble growth was wholly a matter of insufficient nutrition, and found that on adding considerable quantities of cane sugar the growth increased rapidly and became very abundant. About the same time tests with iodine showed that the starch of the potato, even close under the bacterial layer, had been very little acted upon by the organism.

The rather meager growth of this germ on potato now appears to me attributable to its feeble diastasic action, i. e., to its inability to get

from the starch enough food for its normal growth, and I am surprised that this explanation did not occur to me at once. The organism grows fairly well until the small amount of grape sugar present in the potato is exhausted, and thereafter, when thrown wholly upon its own resources, makes only an extremely feeble growth, corresponding to its very feeble diastasic powers. This conclusion rests upon the following experiments:

Iodine Starch Reaction.

My uninoculated potato cylinders when tested with iodine potassium iodide diluted with water, or with iodine crystals dissolved in absolute alcohol to saturation and then diluted with water as required for use, always yielded an immediate bright blue reaction. The starch reaction was also strong after the *Ps. hyacinthi* had been grown on them for several weeks, although there was always evidence of slight diastasic action to be found in the purplish color assumed by some of the grains. The following are transcripts from my notes.

(1) Some fragments of potato scraped from immediately under the yellow slime on a culture 30 days old were put into an old solution of iodine-glycerine. They became black at once, and when crushed out and examined under the microscope were brownish purple—i. e., more brown purple than the starch from a check tube. Tested with alcohol-iodine diluted with fifteen or twenty times its bulk of water, the starch of the potato in the check tubes became pure blue. In the culture, immediately under the yellow slime, most of the starch-bearing cells became purple, but occasionally one was nearly pure blue. Cells deep in the cylinder reacted blue.

(2) On the thirty-first day another tube of the same lot was tested with alcohol-iodine, diluted with thirty or forty times its bulk of water. When this fluid was put on scrapings from a check tube, the reaction was pure blue; when it was put on scrapings from immediately under the yellow slime, the starch reaction was purple and blue purple.

(3) A year previous scrapings were made close under the bacterial layer of a culture 36 days old and tested with iodine potassium iodide. There was a strong blue-black reaction. Under the microscope, however, some of the cells were paler than others, indicating that some of the starch grains had been acted upon slightly.

(4) On the twenty-ninth day a potato cylinder, bearing a typical growth of the yellow slime and uniformly grayed, was broken across the middle and tested with iodine alcohol in water. The middle part of the cylinder reacted blue. The outer part, close under the bacterial layer, gave either a reddish or purplish blue reaction.

No potato cultures of this organism were ever tested which did not give a very decided reaction with iodine. The importance of this fact

will be brought out to best advantage by comparison with *Ps. campestris* or *Ps. phaseoli*, both of which exert on starch a very powerful diastasic action. When either of these germs is grown on potato cylinders in water for 30 days, not simply all of the starch in the surface cells, but also all of that in the deeper parts of the cylinder, is acted upon, and this action is not feeble, but so vigorous and far-reaching that if the whole cylinder is crushed in a large bulk of the iodine water there is either no color reaction whatever, or merely in places a feeble brownish-purple tinge, indicating that all of the starch, or almost all, has been converted.

Growth on Potato with Addition of Cane Sugar.

These cylinders were the ordinary potato cultures in test tubes, to each of which was added 1 gram of cane sugar. At first growth was retarded—e. g., on the fourth day it was slight and white or nearly white. On the thirty-seventh day it was yellow, extended down into the fluid, and was 20 to 25 times as abundant as in the check tubes. The surface was wet-shining, but not smooth, owing to the protrusion of rounded zoogloeæ. On the sixty-seventh day the slime was wax yellow, and covered the whole cylinder, just as *Ps. campestris* or *Ps. phaseoli* would have done without the addition of sugar. The entire culture now looked like shagreen from inequalities in its surface due to the protrusion of rounded masses. The slime was neutral, or at least not alkaline, and the small amount of fluid remaining in the bottom of the tubes was plainly acid to neutral litmus paper. The brown stain of the fluid was less than in the check tubes.

Growth on Potato with Addition of Maltose and Dextrine.

This medium consisted of potato cylinders standing, two-thirds covered with distilled water, in well-plugged test tubes. To each tube was added 100 milligrams of maltose and an equal quantity of dextrine. They were then re-steamed as usual (20 minutes at 100° C., on 3 consecutive days), constituting stock 301. Each tube was inoculated with a large loop of *Ps. hyacinthi* from a well-clouded beef-broth culture 11 days old, check tubes made from the same tuber being held for comparison.

Result.—During the first few days (4 at least) there was not as much growth in the 2 maltose-dextrine tubes as in the 2 check tubes. However, at the end of 24 days (temperature 19° to 25° C.) there was an abundant yellow, wet-shining growth over the whole of the exposed part of the cylinder, down into the upper part of the water, and on the wall of the tube, at least 15 times as much growth as in the check tubes. This growth continued for several weeks.

Growth on Potato with Addition of Diastase of Malt.

This medium consisted of 4 potato cylinders from the same tuber as 301, to each of which was added 500 milligrams of Merck's "diastase of malt absolute." After remaining over night in a water bath at 50° C., these tubes were sterilized by steaming about 20 minutes on 4 consecutive days. Each tube received a large loop from a beef-broth culture 11 days old—the same tube that was used to inoculate the tubes of potato-maltose-dextrine. Two tubes without the disastase were inoculated for comparison. The tubes were kept together in the dark at room temperatures of 20° to 25° C.

Result.—By the end of the third day the check tubes had developed a thin, distinct, yellow growth over nearly the whole of the exposed part (one-third) of the cylinder. The progress of these check cultures from this time on was typical for *Ps. hyacinthi*, there never being any copious growth or any development of the yellow slime under the water. The tubes to which the diastase was added were under observation 55 days. In 3 of them there was never any growth. In the fourth tube growth was retarded until the eighth day (temperatures 20° to 23° C.), on which date a yellow patch 1 cm. square was visible. On the twenty-fourth day the organism had entirely overcome the retarding action of the medium and had made an abundant, distinctly yellow, smooth, wet-shining growth over the whole cylinder down into the water and up on the wall of the tube. This growth was estimated at 50 times that in the check tubes and was greatly in excess of any growth ever before obtained upon potato.

The 3 tubes in which there had been no growth were reinoculated on the twenty-fourth day, using for one a large loop of yellow slime from one of the check tubes, and for each of the other two an equally large loop of slime from the other check tube. This slime was rubbed carefully over the surface. No growth ensued, although the tubes were watched for a month. The fluid in these tubes was neutral to litmus, or very feebly acid when dry, and the restraining influence was therefore attributed to an excess of maltose or dextrine liberated by the diastase. On mashing one of these cylinders in alcohol-iodine water there was no starch reaction whatever nor any red reaction.

Potato Starch in Peptone Water with Diastase.

This medium was prepared in test tubes of resistant glass, using about 1 gram (estimated dry weight) of freshly prepared thoroughly washed potato starch to about 9 c. c. of distilled water which had received 4 per cent of Witte's peptonum siccum. The tubes were then put into the steamer and the starch solidified in a slanting position. Some of these tubes were held as checks, and the surface of the remainder was flooded

with about 1 c. c. of distilled water containing the commercial Taka-diastase. Each tube received 20 milligrams of this diastase, which was allowed to act 4½ hours at 23° C. and then destroyed by steam heat. These tubes were then inoculated from three different cultures of *Ps. hyacinthi*, a beef-broth culture 14 days old, a turnip culture 9 days old, and a carrot culture 9 days old. The fluid loops were streaked; the solid loops were rubbed carefully over the whole slant. The tubes were then kept in the dark at room temperatures ranging from 19° to 23° C.

Result.—In the check tubes at the end of 48 hours there was a slight to very slight growth. On the eighteenth day from one-fourth to three-fourths of the slant surface in these tubes bore a thin bright yellow growth, which never increased much. The development of the germ in the tubes which received the diastase was plainly different. At the end of 48 hours the growth was distinctly yellow and much better than in the check tubes. On the ninth day the principal difference was still the amount of growth which was several times that in the check tubes. On the eighteenth day the growth was dirty yellow, wet-shining, and copious, i. e., at least 10 times as much as in the check tubes. The difference in color was very decided. The slime in the check tubes was pure yellow; that in the others was dirty yellow, verging into brownish. The tubes were now thought to be rather too dry, and 2 c. c. of sterile Potomac River water was pipetted into each one, the result being a somewhat increased growth. On the forty-fourth day the growth in the check tubes was still feeble and much less than in the tubes which received the diastase. The substratum of the latter had become brownish-white with the merest trace of pink in it. The same stain appeared in the check tubes, but was much feebler.

Tubes of *Ps. campestris* and *Ps. phaseoli* yielded some instructive comparisons. In the check cultures, on the sixteenth day, the growth of these two germs was at least 20 times as abundant as that of *Ps. hyacinthi*. On the seventy-third day, in the check tubes, the layer of *Ps. hyacinthi* was still feeble, and was still distinctly yellow; that of *Ps. campestris* and *Ps. phaseoli* was 100 times as abundant and had lost all of its pure yellow color, this having changed into a decided brown. The starch in the check tubes of the hyacinth germ was as firm, elastic, and insoluble as when first inoculated, and was but little stained; that in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli* was gray, soft-mushy, and soluble in water. Tested in alcohol-iodine diluted with 50 volumes of distilled water, the check cultures of *Ps. hyacinthi* gave a strong starch reaction; those of *Ps. campestris* and *Ps. phaseoli* gave no color reaction whatever. One culture of each was also tested with Soxhlet's solution for the presence of reducing substances. *Ps. campestris* and *Ps. phaseoli* each reduced 25 c. c. of the standard solution of copper sulphate (34.639 grams of c. p. $CuSO_4$ 5 H_2O

in 500 c. c. of H_2O). The tests were made in the usual way with 5 c. c. portions of the copper solution and 5 c. c. of the alkaline solution in 40 c. c. of distilled water, boiling 2 minutes in white porcelain capsules. The check culture of *Ps. hyacinthi* was estimated to have reduced only a small fraction of 1 c. c. of the copper solution. On settling there was only a little red precipitate and the fluid was still quite green-blue, so that perhaps not more than one one-thousandth of the starch was converted. The cabbage and the bean germ grew as well, or very nearly as well, on the peptone-potato starch without the diastase as with it, the cultures looking much alike.

Inasmuch as the Taka-diastase contained a trace of some reducing substance and the peptone in water was able of itself to nourish the hyacinth germ for a time, it was thought best to repeat this experiment, using solutions of mineral salts, sodium asparaginate and ammonium lactate in place of the peptone, the same kind of starch, and a Taka-diastase, reprecipitated for me by Dr. John M. Francis.

Nutrient Starch Jelly No. 1.

This medium was prepared from Uschinsky's solution, substituting potato starch for the glycerine. My method of preparing this medium and the following one need not be given here, as it has been published in Proceedings of the American Association for the Advancement of Science, Boston meeting, 1898, page 411, and in Centralblatt für Bakteriologie, 2. Abt., Bd. V, page 102. All that is necessary to say is that each tube contained from 5 to 7 c. c. of the solution and 1 to 1.5 grams of the dry starch. After the starch had set and was ready for use the check tubes were counted out and the slant surface of the jelly in each of the others was flooded with 1 c. c. of distilled water containing exactly 20 milligrams of the diastase. These tubes were then put into the thermostat at 34° C. for 1½ hours, and afterwards the diastase was destroyed and the tubes sterilized in the usual way, i. e., by steaming for a few minutes on 3 consecutive days.

Before using, the diastase was carefully tested for the presence of reducing substances and found to be entirely free. This diastase likewise gives no blue reaction with guaiac resin and hydrogen peroxide. The starch jelly was also tested in the same way, using Soxhlet's solution, and was found to be entirely free from reducing substances. On the contrary, bits of starch jelly from the tubes which had been treated with the diastase gave an immediate rusty precipitate when dropped into the boiling fluid.

Three tubes of this medium were inoculated, along with 3 check tubes. These 6 tubes were divivided into 3 lots, each group being inoculated from a separate culture. All were kept in the dark at room temperatures, which ranged from 19° to 25° C. during the first 2 weeks and then from 25° to 34° C. (mostly 25° to 29°).

Result.—Two of these groups of tubes failed to catch and were reinoculated later so that the first group will be considered by itself.

(1) These 2 tubes were inoculated in the same way from a fluid culture 32 days old. During the first 18 days there was no trace of color or sign of growth in the check tube. On the twenty-seventh day there was a slight growth with feeble yellowing of the surface, but careful scrutiny was necessary to detect it. On the thirty-fifth day a slight increase of growth was noted. The starch had not dried out much and the whole of it was still bluish white, indicating that there had been no considerable diastasic action. The streak was very thin, very pale yellow, did not hide the substratum, and had no well-defined margins. On the sixty-second day there was decidedly more growth, the whole surface being covered with a thin, distinctly yellow, smooth, homogeneous, wet-shining layer. The body of the starch still preserved its bluish white luster and retained its water well. The amount of growth in this tube after 62 days was not greater than that present in the other tube at the end of 5 days. In the tube which received the diastase there was, on the fifth day, a distinct but not very copious growth, covering about two-thirds of the slant surface. On the twelfth day there was an abundant bright yellow growth covering the whole surface and affording a striking contrast to the check tube. This contrast continued for some time, the difference in the 2 tubes on the fourteenth day being shown in figs. 15 and 16 of the plate accompanying bulletin 26 of this Division. The color was approximately Ridgway's canary yellow (VI–12). On the twenty-seventh day the slime was still bright yellow, and the amount of growth was estimated at 200 times that in the check tube. On the thirty-ninth day there was still no brown stain.

(2) After 8 days the other 4 tubes were reinoculated copiously over the whole surface with yellow slime taken from the culture just described. They were under the same conditions as to light and temperature, the greatest difference between these and the preceding being the enormous number of germs used in making the inoculation.

Result.—The 2 check tubes behaved alike. On the fourth day there was a trace of yellow growth at the bottom of the slant, but it was feeble, and was visible on not more than one-fiftieth of the whole surface. At this time the tubes which received the diastase showed an abundant bright yellow growth over the whole surface, a growth several hundred times as abundant as that in the check tubes. On the sixteenth day, in the check tubes, there was only a feeble growth of 9 or 10 square millimeters. This growth was bright yellow, but it was not one one-hundredth as much as in the tubes which received the diastase. On the twenty-seventh day, in the check tubes, the growth had doubled, but the substratum was hidden only over a few square millimeters, and the

ratio of growth in the 2 sets of tubes was still about the same, viz, 1:100. The starch was still bluish white. On the twenty-seventh day, in the tubes which received the diastase, the growth covered the whole surface of the slant (800 to 900 sq. mm.) with a smooth, homogeneous, wet-shining, canary yellow layer, which was abundant enough to hide the substratum. There was a trace of pink in the starch, but no brown stain. On the thirty-fifth day the starch jelly was removed from one of the check tubes. It was as firm and elastic as when first prepared. On breaking it into fragments and throwing it into boiling Soxhlet's solution (5 c. c. standard $CuSO_4$ 5 H_2O solution; 5 c. c. standard alkaline solution; 40 c. c. distilled water) and continuing the boiling 3 minutes, the fluid was as blue as at the beginning, and the only precipitate of copper oxide was an extremely slight one restricted to those fragments of the jelly which were immediately under the bacterial layer. Certainly not more than one one-thousandth of the starch was converted.

(3) The experiment just described was repeated 3 months later in the warmer weather of midsummer. A new stock of the medium was prepared and in this case each tube received 2 gr. of the dry potato starch and 8 c. c. of the nutrient mineral solution. Instead, however, of converting the starch with diastase, the carbon food was supplied by the addition of various sugars, alcohols, and gums. No mention will be made here of anything but the check tube and a tube of the same stock fortified by the addition of 500 mg. of a dextrine, which contained a substance reducing Soxhlet's solution but no amylodextrine and no substance reducing Barfoed's reagent. Both tubes were inoculated at the same time and in the same way; i. e., each with a large loop of yellow slime from a fructose-agar culture 17 days old, but still in excellent condition owing to its having grown slowly on the start. The tubes were kept in a dark closet at room temperatures ranging from 25° to 32° C. (30° to 32° during the first 5 days). Tubes of *Ps. campestris* and *Ps. phaseoli* were also inoculated at the same time and kept under the same conditions.

Result.—In the check tube of *Ps. hyacinthi* there was no visible growth during the first 48 hours. On the third day there was a very slight growth (barely visible), and the bluish white translucent appearance of the starch remained unchanged. In the tube which received the dextrine, growth was visible in 48 hours, but it was still feeble on the third day; i. e., growth was retarded. On the third day, in the check tubes of *Ps. campestris* and *Ps. phaseoli*, there was 20 times as much growth, and the starch jelly under the slime, to a depth of 2 mm., was changed to a dead, opaque white. Returning to the hyacinth germ, there was on the seventh day, in the check tube, a very thin, pale yellow streak or film down the middle of the slant. In the tube which received the dextrine the whole surface was covered by a

thin distinctly yellow layer; i. e., there was several times as much growth as in the check tube, but there was no visible diastasic action. The growth of *Ps. phaseoli* on the check was now at least 100 times as abundant as that of *Ps. hyacinthi* on the same medium.

On the starch jelly with addition of the dextrin *Ps. campestris* and *Ps. phaseoli* both made a good growth. On the seventh day *Ps. campestris* covered the whole surface of the long slant to a depth of 1 to 3 millimeters with a semifluid, smooth, wet-shining slime, and the diastasic action now involved nine-tenths of the starch. The conversion of the starch was clearly visible, proceeding slowly and uniformly from the surface of the slant inward. There was a distinct line of demarcation between the converted and unconverted starch. The latter was bluish white, opalescent, translucent, firm, elastic, insoluble; the former was dead white, opaque, soft, inelastic, and soluble in water on gentle shaking. This part gave no color reaction whatever on adding iodine water. On washing it all out the unchanged one-tenth in the bottom of the tube was seen to have preserved the shape of the original slant, and on adding the iodine water it became bright blue. In the corresponding tube of *Ps. phaseoli* the growth at this time appeared to be equally as good, but only about two-thirds of the starch was converted. The diastasic action proceeded from the surface inward in the same regular manner, the line of demarcation between converted and unconverted starch was equally sharp, and the converted portion had all the peculiarities recorded for that acted on by *Ps. campestris*. A fragment of this soft white starch as big as two peas was stirred up in 5 c. c. of the very sensitive pale brown alcoholic iodine water, but no color reaction could be obtained. This changed starch included all of the outer 5 or 6 millimeters of the slant; on filling the tube part full of water and shaking gently all of it dissolved readily, leaving in the bottom a translucent, bluish white, insoluble, miniature slant, which immediately reacted bright blue on pouring in the same iodine water. These experiments show that the presence of albuminoids is not necessary for the production of the diastasic ferment and also that it is excreted by these two species in the presence of an abundance of readily assimilable food.

On the twelfth day, in the check tube of *Ps. hyacinthi*, the thin, pale yellow growth had extended over most of the slant surface, but it was still not one-hundredth part as abundant as in the corresponding tube of *Ps. phaseoli*, and there was no evidence of any diastasic action, whereas in the latter more than nineteen-twentieths of the starch had been digested. In the tube which received the dextrin, *Ps. hyacinthi* had made, on this date, a good, bright yellow but rather dry growth over the whole surface. On the thirtieth day, in this same medium, there was a plentiful, smooth, wet-shining, bright yellow slime over the whole surface, i. e., growth enough to hide the sub-

stratum, but no brown stain, no decided smell, and no ocular evidence of any diastasic action. The germs were carefully scraped off and iodine water poured into the tube, whereupon there was an immediate and general blue reaction, showing that very little of the starch had been changed. This shows clearly that increased growth does not necessarily imply any increased secretion of the diastasic ferment. The check tube could not be compared owing to a contamination.

(4) A few days later another check tube was inoculated and a similar feeble growth ensued. A tube containing 500 milligrams of dextrin, which was inoculated for comparison, gave a much better growth. On the third day the whole surface of the slant in this tube was covered by a thin, distinctly yellow, dry layer, and there was no visible diastasic action. On the twelfth day the growth was smooth, wet-shining, bright yellow, and about 8 times as abundant as in the check tube. There was also a decided diastasic action, involving the outer 5 millimeters of the starch. This result contradicts the preceding experiment with dextrin and is probably attributable to the action of some undetected, intruding organism (see p. 64).

Nutrient Starch Jelly No. 2.

The nutrient solution used in preparing this medium differed from the preceding by addition of sodium sulphate; by a considerable reduction of the magnesium sulphate and calcium chlorid; by a slight reduction of the sodium chlorid, sodium asparaginate, and ammonium lactate, and by a slight increase of the dipotassium phosphate (for exact composition see *loco cit.*). Each tube received exactly 10 c. c. of this solution and 2 grams of dry potato starch free from any trace of sugar. For comparison a culture was laid at the same time on starch jelly No. 1, containing 2 grams of the same starch and 10 c. c. of the glycerin-free Uschinsky. The slant surface of each substratum was inoculated in the same way, carefully and very copiously, with bright yellow slime from a starch-jelly culture 17 days old. The tubes were kept in a dark place at room temperatures ranging from 21° to 31° C. (most of the time below 28°).

Result.—On the fifth day there was a feeble, bright yellow growth, much alike in each tube, and no visible diastasic action. On the eleventh day there was a thin, bright yellow growth over nearly the whole surface—i. e., a considerable increase of growth, but still no diastasic action. Both tubes were much alike, but there appeared to be slightly more growth in starch jelly No. 1. On the twenty-fourth day the growth in starch jelly No. 2 had increased but little. This growth was wet-shining and distinctly yellow, but so feeble that the substratum was not hidden; there was no brown stain in the substratum, and no visible diastasic action. In starch jelly No. 1 there was distinctly more growth, but no visible diastasic action. An intruding colony

(the product of a spore which passed through the sterilizing oven uninjured) had come to the surface, and I suspected that sugar liberated by this colony had diffused through the substratum and stimulated the growth of *Ps. hyacinthi*. On the thirty-fifth day there was no increased growth and no visible diastasic action in jelly No. 2, but in jelly No. 1 the bright yellow growth was 3 or 4 times as abundant and was now clearly attributable to diffusion of sugar, or some other assimilable substance, liberated by the intruding organism. There was no visible diastasic action except in the starch immediately around where this white colony had come to the surface. The effect of the growth of this intruder was most clear cut and interesting.

For comparison with these two tubes a culture was laid at the same time on starch jelly No. 2 with addition of 500 milligrams of dextrin. The organism grew well on this substratum, making 4 to 6 times as much growth as in the check tubes. On the twenty-third day, when last examined, there was an excellent growth and had been for 3 weeks, but there was no visible diastasic action.

Ps. phaseoli was very pale and made a much less abundant growth on nutrient starch jelly No. 2 (made with the modified Uschinsky's solution minus the glycerin) than it did on potato, or than did *Ps. campestris*. In Uschinsky's solution, on the contrary, it was yellower and grew rather better than *Ps. campestris*.

Hyacinth Starch Jelly.

This was made by adding 1 gram of dry sugar-free hyacinth starch, obtained from bulbs, to 5 c. c. portions of Uschinsky's solution. Three tubes were prepared, to one of which was added 500 milligrams of cane sugar. The tubes were steamed 2 hours on each of 3 consecutive days at 91° C., this low temperature being obtained by putting the tubes in the top of the steamer with the vents left open. The tubes were inoculated with *Ps. hyacinthi* very copiously in the same manner soon after the third steaming from a starch jelly culture 7 days old. They were kept together in a dark place at room temperatures ranging from 15° to 26° C.

Result.—At the end of 48 hours (temperature, 21° to 22° C.) growth was visible in each tube. At the end of the fourth day the 2 check tubes were alike, the whole surface of the long slant being covered with a very thin, distinctly yellow growth. There was, however, no visible diastasic action, the organism behaving on hyacinth starch exactly as on potato starch. In the tube which received the cane sugar there was 4 or 5 times as much growth as in either of the check tubes. This growth was bright yellow and covered nearly the whole surface of the slant, but there was no visible diastasic action, the increased growth being due to the presence of the cane sugar. A little later this tube was accidentally broken. The check tubes were

under observation for an additional 24 days, during which time a great change took place in one of them, the growth increasing tenfold. This increased growth of the organism was due to no diastasic action of its own, but to the diffusion of maltose or dextrin liberated from the starch by some buried, slow-growing, white, starch-converting colonies, which originated from spores that found their way into the starch during its preparation and which passed through the steamings uninjured.

The foregoing conclusion is also supported by the fact, already set forth, that *Ps. hyacinthi* grows well on a variety of crude vegetable substances rich in sugar. That this feeble diastasic action partially accounts for the feeble parasitism admits of little doubt. Probably its feeble cytohydrolytic action and its strict aerobism are also restraining influences.

AEROBISM.

As already noted, the buried colonies of *Ps. hyacinthi* in plate cultures grew slowly, and those deepest in the layer of agar or gelatin remained smallest. In the stab cultures also the bacteria gradually faded out in the depths, making much the best growth near the surface. The additional results bearing on the inability of this germ to grow in the absence of free oxygen are thrown together in the following paragraphs.

Fermentation Tubes.

The form of tube used in my laboratory is that devised by Dr. Theobald Smith and made by E. Greiner, of New York.[1] This, by reason of its size and shape, the writer has found more satisfactory than several other sorts he has tried.

First is a table, which sets forth the results obtained with fermentation tubes in 1897. The tubes were filled with distilled water containing 1 per cent of Witte's peptonum siccum and 1 per cent of the sugar or other substance to be tested.

[1] The fermentation tube, with special reference to anaerobiosis and gas production among bacteria. The Wilder Quarter Century Book, Ithaca, N. Y., 1893, p. 187.

TABLE IV.—*Results of growth of Ps. hyacinthi in fermentation tubes. Inoculated Feb. 12.*

Substances tested in the fermentation tubes in distilled water.		Results obtained.					
		Clouding.		Gas production.	Reaction to neutral litmus paper.		Duration of experiment. (Days.)
Nitrogen.	Carbon.	Open end and outer two-thirds or three-fourths of the U.	Closed end.		Acid.	Alkaline.	
Peptone (1 per cent Wittes's peptonum siccum).	Grape sugar (1 per cent Merck's c. p. anhydrous).	Feb. 15 (third day). Well clouded; hundreds of tiny flecks (zoogloeæ), which show a decided preference for the upper layers of the liquid, but settle on jarring gently. Feb. 19. Zoogloeæ continue. Feb. 23. Sharp line of demarcation in the U (three-fourths clouded). Mar. 1. Moderately cloudy; no pellicle; slight precipitate. Mar. 12. Moderately cloudy; no pellicle; considerable pale yellow precipitate. Mar. 20. No change.	Perfectly clear	No gas.	No acid.	Slowly increasing alkalinity.	38
Do	Fruit sugar (1 per cent Schering's diabetine).	Feb. 15 (third day), 19 and 23. In all respects like the preceding. Mar. 1. Like the preceding, but a trifle more precipitate. Mar. 12. Moderately cloudy; clouding involves outer three-fourths of U, with sharp line of demarcation; no pellicle; considerable pale yellow precipitate. Mar. 20. No change.	do	do	do	do	Do.
Do	Cane sugar (1 per cent white, granular, commercial).	Feb. 15 (third day). Like preceding. Feb. 19. Same, except no zoogloeæ visible. Feb. 23. Sharp line of demarcation in the U. Mar. 1. About as cloudy as preceding; a slight precipitate; sharp line of demarcation continues. Mar. 12. Exactly like the two preceding. Mar. 20. No change.	do	do	do	do	Do.
Do	Milk sugar (1 per cent c. p. Once recrystallized in laboratory Div. Chem., U. S. Dept. Ag.).	Feb. 15 (third day) and Feb. 19. Same as tube of grape sugar. Feb. 23. Sharp line of demarcation in the U. Mar. 1. Like tube of grape sugar. Mar. 12. There is now noticeably less precipitate than in the tubes containing the grape, fruit, or cane sugar; otherwise the same. Mar. 20. No change.	do	do	do	do	Do.
Do	Maltose (1 per cent c. p. from Div. Chem., U. S. Dept. Ag. Tested with polariscope and found to give proper rotation.)	Feb. 15 (third day). Like the tube of grape sugar. Feb. 19. Like the tube of grape sugar. Feb. 23. Sharp line of demarcation in the U. Mar. 12. Moderately cloudy; no pellicle; slight precipitate; precipitate plainly less than in tubes containing the grape, fruit, and cane sugar; much like that in tube containing the milk sugar. Mar. 20. No change.	Feb. 19. Clear. Feb. 23 Very feebly clouded, perhaps one-twentieth or one-thirtieth as much as in open end. Clear, except on very close inspection. Possibly the clouding may be due to some accidental tilting of the tube.	do	do	do	Do.

			Mar. 1. No change; doubtful if any growth. Mar. 12. Perfectly clear.				
Do	Dextrin (1 per cent commercial 10 times precipitated with alcohol in laboratory of Div. Chem., U. S. Dept. Agr. This dextrin contained a substance reducing Soxhlet's solution, but did not redden with iodine and did did not reduce Barfoed's solution.)	Feb. 15 (third day), Like the tube of grape sugar except that the zooglœae can not be made out clearly. Feb. 19. Hundreds of tiny zooglœae are now present. Feb. 23. Sharp line of demarcation in the U. Mar. 1. Like tube of grape sugar, but more cloudiness and more precipitate. Mar. 12. Quite cloudy, more so than in any of the preceding tubes and very decidedly more than in the tubes of milk sugar or of maltose; no pellicle; copious, pale yellow precipitate; this dextrin seems to be a particularly good food. Mar. 20. No change.	Perfectly clear	do	do	do	Do.
Do	Mannitol (1 per cent Merck's c. p., in which the polariscope showed no contamination).	Feb. 15 (third day). Like the first except no zooglœae visible. Feb. 23. Sharp line of demarcation in the U. Mar. 12. No pellicle; no visible precipitate; clouding slighter than in tube of grape sugar, i. e., like that in tubes of milk sugar and of maltose. Mar. 20. No change.	do	do	do	do	Do.
Do	Glycerol (1 per cent Schering's twice distilled, sp. gr. 1.260, at 30.8°, Bé. strictly c. p.).	Feb. 15 (third day). Like tube containing the grape sugar, but no zooglœae visible. Feb. 19. Still no zooglœae, otherwise like tube of grape sugar. Mar. 12. Moderately cloudy, only a slight precipitate. Mar. 20. No change.	Feb. 19. Clear. Feb. 23. Very faintly clouded (like tube of maltose). Not yet certain what this faint cloudiness means. Mar. 1. As before, probably to be recorded as clear. Mar. 12. Perfectly clear.	do	do	do	Do.

Each of these tubes was inoculated February 12 with one loop from a beef broth culture made February 5. This culture was well clouded and becoming moderately turbid from the presence of numerous small zooglœae. It also contained a moderate amount of yellow precipitate. The tubes were very clear when inoculated, and perfectly sterile, the third steaming having taken place some weeks previous. The fluid in each tube was feebly alkaline to litmus when inoculated. The cultures were kept at living-room temperatures (20° to 23° C.). February 19 the cultures were first tested with litmus (the best neutral litmus paper procurable). The fluid in each was plainly alkaline (much more so than on the start). March 1 the fluids were again tested. All were alkaline to neutral litmus paper. March 12 the cultures were again tested. Each was plainly alkaline, although not strongly so. The blue color faded out when the paper dried. If any acid was formed it was masked by the alkali originally present in the tubes and by that produced during the growth of the organism.

The above results were obtained in 1897. In 1899 additional fermentation-tube experiments were instituted with the following results:

(1) One of the fluids used was a 1:2 nonpeptonized beef bouillon (stock 382) rendered neutral to phenolphthalein with sodium hydrate and deprived of its muscle sugar by growing *Bacillus coli* in it over night. It was then cleared by passing it through a Chamberland filter. The following substances were tested in this bouillon: Grape sūgar, cane sugar, and galactose (3 tubes of each). Each tube contained 5 per cent of the sugar to be tested except those with the grape sugar which contained 2.8 per cent. The inoculations were made February 2 and the experiment was closed March 4. The tubes of grape sugar and cane sugar were all well clouded (in the bowl and outer two-thirds of the U) on the fifth day, with exception of one of the grape-sugar tubes which was then only very feebly clouded, but was well clouded 2 days later. In each case the closed end of the tube and the inner one-third of the U remained clear until the end of the experiment. The reaction to litmus was watched carefully. The fluid in the bowl of each of the tubes was plainly alkaline to litmus paper (wet or dried) on the ninth, fifteenth, and twenty-third days. On the thirtieth day in each tube, whether of grape sugar or cane sugar, the litmus reaction was distinctly different. The tests were made with two freshly prepared sensitive litmus papers, the one purplish red, the other pale lavender blue. The fluids now blued the purplish red paper slightly and at the same time reddened the bluish paper. The contrast in each case to inoculated check tubes of the plain bouillon (which were now intensely alkaline and blued both papers) was striking. The only conclusion I could come to was that a definite but small amount of acid had been formed slowly from the grape and the cane sugar. In comparison with plain bouillon these

sugar bouillons stimulated growth. No gas was formed. The growth in each was typical for *Ps. hyacinthi.*

In 2 of the 3 tubes of galactose *Ps. hyacinthi* refused to grow, and in the third clouding did not appear until after the seventh day. On the ninth day the fluid in the bowl and outer two-thirds of the U was feebly clouded. After a time there was an abundant yellow growth on the open end of the tube, but the closed end remained clear throughout the experiment. The fluid, as we have seen, was neutral to phenolphthalein (strongly alkaline to litmus) on the start. It was still strongly alkaline to litmus on the ninth day; on the fifteenth day it was moderately alkaline. On the twenty-third day it was neutral to litmus or nearly so, but so was an uninoculated tube. On the thirtieth day the fluid was distinctly acid, even to the purplish red paper. No gas was produced. The cloudy fluid was now pipetted from the open end of the bulb into a clean test tube and reduced by boiling to one-third its original volume. Moistened litmus paper was reddened in the vapors which first came off (CO_2?). Afterwards there was no reddening of the litmus paper in the steam and the concentrated fluid was more acid than before.

The fact that the organism failed to grow in two of the tubes and was retarded in the third was attributed to the effect of a soluble brown substance which appeared in the tubes as a result of the 3 steamings which followed the addition of the galactose.

(2) Absolute ethyl alcohol was also pipetted into 4 tubes of the same stock. Two of the tubes received 2½ per cent and two 5 per cent of this alcohol. Each tube was then inoculated with two 3 mm. loops from fluid cultures 13 days old (tubes 1 and 2, January 20, 1899). This experiment was suggested by the results obtained with Sharp and Dohme's litmus solution in milk. In one of the 5 per cent alcohols the organism failed to grow. In the other 3 tubes clouding occurred on the fifth to the seventh day; i. e., growth was retarded very decidedly. The tubes never became heavily clouded; growth ceased early and the closed end remained clear (30 days). The fluid was plainly alkaline to litmus at the beginning and on the ninth and the fifteenth days. On the latter date the appearance of the cultures was that of simple toleration of the alcohol rather than of any use of it for growth. The alkalinity in one of the 2½ per cent tubes on the fifteenth day was rather feeble; i. e., much less than in an uninoculated tube or than in inoculated tubes of the simple bouillon. On the twenty-third day the fluid had settled clear and was feebly acid to litmus. On the thirtieth day the fluid (in each tube) was clear and was distinctly acid to both the litmus papers. No gas had formed. The precipitate was distinctly yellow but scanty; i. e., there was only about one-twentieth to one-fiftieth as much as in the tubes of simple bouillon and about one one-hundredth as much as in the tubes of grape sugar and cane

sugar. The least growth was in the 5 per cent alcohol. Evidently the acid which was formed inhibited growth, although it did not immediately kill all of the organisms. This was determined by making 6 cultures from the 5 per cent alcohol on the twenty-third day (2 carrot, 2 potato, and 2 coconut cultures—1 loop for each). The organism grew in all these tubes, but its development was slow. It was not visible in any of them on the fourth day. The yellow growth appeared in 5 of these tubes on the sixth day and in the sixth tube a day or two later. A fact which shows the remarkably slow diffusion of the acid is that the fluid in the closed end of the tubes (2½ per cent alcohol) remained alkaline while that in the open end became acid. On the thirtieth day, in 1 tube, the fluid in the bowl was "distinctly acid to the blue paper and also to the pale red paper;" in the other it was "strongly acid to both red and blue papers." Nevertheless, when the contents of these 2 tubes was poured out into a clean test tube and thoroughly mixed it was no longer acid to either paper, but had become slightly alkaline; i. e., not enough acid was produced in the open end of the tube to neutralize the sodium hydrate in the 25 c. c. of fluid (25 c. c. of $\frac{N}{1}$ NaOH per liter). This fluid was then reduced one-half by boiling, but no acid vapors appeared in the steam.

(3) The experiments with glycerol and maltose were repeated to see whether the faint clouding which finally appeared in the closed end, in the experiments of 1897, should be attributed to facultative anaërobism or only to some accident. The stock used was a 1:2 slightly alkaline non-peptonized sugar-free beef bouillon (No. 450).[1]

To this was added 2 per cent of Schering's twice distilled c. p. glycerin in the one case and 2 per cent of Merck's c. p. maltose in the other. The experiments were carried through in duplicate. Having been on the shelf 15 days since the last sterilization, the tubes were resteamed for 20 minutes but no air bubbles appeared. Each tube was then inoculated with one 3 mm. loop from a cloudy broth culture 3 days old. The observations were continued 23 days.

Result.—The tubes of glycerin bouillon clouded in the bowl and outer three-fourths of the U on the second day, but remained entirely clear in the closed end during the whole time. The glycerin gave no increased clouding, i. e., not more than the simple bouillon. The line of demarcation in the U remained sharp. The fluid was slightly alkaline to litmus when inoculated and was neutral to feebly alkaline at the close of the experiment.

The maltose bouillon was feebly clouded in the bowl and outer three-fourths of the U on the second day. The line of demarcation in the U was sharp on the third day. On the seventh day the bowl and outer three-fourths of the U were uniformly and well clouded. This clouding was decidedly more than in the corresponding tubes of glycerin

[1] Freed from muscle sugar by *B. coli* and clarified with white of egg.

bouillon. The closed end and inner one-fourth of the U were still perfectly clear. On the twelfth day the line of demarcation in the U was less distinct and there was a faint haze in the lower part of the closed end (3 cm.). On the twenty-third day the faint haze had involved the whole of the closed end, but had not become any denser, i. e., the clouding in the closed end was not one one-hundredth part that in the open end. The fluid was feebly alkaline at the beginning of the experiment and was decidedly acid (to neutral litmus paper) at the close, i. e., the reaction was in marked contrast to that of the glycerin bouillon. Both tubes of the maltose bouillon behaved alike. They had been protected from jarring and inequalities of temperature, and steaming for 50 minutes at the close of the experiment did not cause the formation of any air bubble in the closed end. This very feeble clouding in the closed end after the second week would seem therefore to be due either to some contaminating substance in the maltose or else to that substance itself.

(4) The nitrate bouillon (stock 474) was also tested in fermentation tubes. Two tubes were inoculated from solid cultures 7 days old. Both clouded on the second day; both remained entirely clear in the closed end and inner one-fourth of the U until after the eighth day. On the fourteenth day both were feebly clouded in the whole of the closed end. No gas was formed and the fluid remained strongly alkaline.

On steaming these two tubes a bubble appeared in the closed end of each, and the feeble clouding was consequently attributed to growth stimulated by the presence of air absorbed from the open end.

The closed end of fermentation tubes filled with the following substances and inoculated with *Ps. campestris* remained entirely free from clouding: Potato broth; cabbage broth; cauliflower broth; peptone water with grape sugar, fruit sugar, cane sugar, milk sugar, galactose, maltose, dextrin, and glycerin. The open end clouded.

Dibasic calcium phosphate added in 5, 10, 20, and 30 milligram doses to test tubes holding 10, 15, and 20 c. c. of a peptone water containing grape sugar and glycerin, doubled the growth of *Ps. campestris*. Other species were not tried. This fluid was then tested in fermentation tubes. The calcium salt stimulated growth in the open end, but the closed end remained clear for three weeks. Afterwards there was clouding. This stock consisted of 200 c. c. of filtered Potomac water, 2 grams of Merck's c. p. anhydrous grape sugar, 4 c. c. of Schering's glycerin, and 2 grams of Witte's peptonum siccum; the whole dried out one-half by long standing and diluted with three times its bulk of distilled water before filling into the tubes and adding the phosphate.

Dibasic sodium phosphate used in the same stock also favored the growth of *Ps. campestris*.

From the above account it will be seen that in various ways the behavior of *Ps. hyacinthi* in fermentation tubes closely resembles that

of *Ps. campestris*. *Ps. phaseoli* has not been tested so extensively but reacts in the same way, so far as tried, i. e., it produces no gas and is strongly aerobic. *Ps. stewarti* produces no gas and appears to be strictly aerobic, but is able to get along with a relatively small amount of air. A small amount of some non-volatile acid or acids appear to be produced by it from grape sugar, cane sugar, galactose, and mannitol, but not from glycerol.

Growth in Nitrogen.

The tests were made in U tubes holding 250 c. c. and open at each end. Two very short cotton-plugged test tubes containing the freshly steamed culture medium were inoculated with *Ps. hyacinthi* and thrust, one above the other, into one arm of the U tube, which was then tightly closed with a soft rubber stopper and plunged, for greater security, into a beaker of glycerin. Into the other arm was thrust quickly a longer test tube filled with a mixture of pyrogallic acid, caustic potash, and water. This end of the tube was then plunged into a beaker of mercury and held down until the absorption of oxygen equalized the pressure and enabled it to remain down of its own weight—a period of some hours. The following experiments were tried in these tubes:

(1) The first experiment was with cylinders of freshly prepared coconut, a medium on which this organism was known to grow without retardation. Four tubes were inoculated. Two received each one loop of yellow slime from a solid culture 7 days old, which slime was rubbed carefully over the whole surface. Two received each two loops of fluid from the bottom of a potato (?) culture 7 days old, after shaking. One tube of each set was held as a check. The other 2 tubes were put into one arm of a U tube the other end of which received a tube holding 2 grams of pyrogallic acid and 25 c. c. of 13 per cent caustic potash water. The room temperature during the experiment ranged from 17° to 26° C. The oxygen was gradually absorbed and the tubes remained exposed to the nitrogen for 15 days.

Result.—In 48 hours from the time of inoculation the check tubes showed a good growth. On the eighth day the check cylinders were covered with an abundant, smooth, wet-shining, canary-yellow growth. In each tube there was at least 6 sq. cm. of this growth. During the same time, in the tubes exposed to the nitrogen, there was no visible growth.

On the fifteenth day the mercury seal was broken and the tubes were taken out and examined more critically. One tube showed no growth whatever and the other an extremely slight pale-yellow growth, best seen with a hand lens, and aggregating not over one-fourth of 1 sq. mm., i. e., not more than might have grown around one of the coarser fragments of the inserted slime before all of the oxygen was absorbed. At this time the contrast with the checks was very

striking. The tiny bacterial mass referred to contained no chains, no spores, and no involution forms. It consisted of slender rods, single or in pairs and very short, as if not now dividing. Exposure of these rods for 10 minutes to a temperature of 74° C. falling to 60° C. killed all of them.

The unexpected feature of this experiment was that after removal to the air growth did not appear in these tubes as soon as it did in the check tubes; in other words, the sojourn in the nitrogen seemed to have exerted an injurious influence. One of the tubes (that inoculated from the solid culture) showed a slight growth at the end of the third day, the other one not until the fifth day. Five days after removal from the nitrogen the bacteria in one tube had made about as much growth as the check tubes made in 48 to 60 hours. In the other tube they had made a thin pale yellow growth covering not more than 1 sq. cm.—i. e., not more than one-tenth as much growth as the check tube made in the same time. In the course of another 3 or 4 days the bacteria in both tubes made an abundant bright yellow growth.

Ps. stewarti tested at the same time behaved in the same way. At the end of 15 days, when the seal was broken, there was no yellow precipitate or visible slime, colored or colorless, in either tube. The two check tubes showed a distinct growth in 48 hours, and continued to grow in a typical way. On the contrary, there was no visible growth in either tube on the fourth day after removal from the nitrogen. On the fifth day in the tube which was inoculated from a solid culture there was a slight yellow growth over a few square millimeters. In the other tube no growth was visible until the eighth day after the removal, and then it was scanty. This cylinder stood in one-half c.c. water and was still moist. Two days later there was a good growth on both cylinders.

(2) The stock in the second experiment with *Ps. hyacinthi* consisted of 6 tubes of white turnip. Each of 3 was inoculated with one loop of a very cloudy beef-broth culture 6 days old. Each of the other 3 was inoculated with one loop of very cloudy fluid from the bottom of a young bright yellow and very vigorous culture on coconut after prolonged shaking. Two of the tubes were held as checks. The other 4 were put into 2 U tubes in the way already described. In each case a test tube (capacity 25 c. c.) packed nearly full of pyrogallic acid was then filled with 6½ per cent caustic potash water and immediately thrust into the other arm of the tube, which was then plunged into the mercury. By the end of 24 hours, and probably sooner, the absorption of the oxygen was complete—i. e., there was no farther rise of the mercury or change in the color of the pyrogallic acid.

Result.—In one of the check tubes growth was plainly visible on the third day, in the other not. On the sixth day in one check tube there was an abundant smooth, wet-shining growth over the whole cylinder

out of the water; the fluid was also heavily clouded, and there was considerable pale yellow precipitate. In the other check tube growth was not so abundant, but about 3½ sq. cm. of the slant surface was covered with a smooth, wet-shining, pale yellow growth.

The tubes were removed from the nitrogen on the fifteenth day. In none of the 4 had there been any growth whatever, although there was an abundance of moisture in each. Moreover, in none of them did any growth subsequently appear (17 days).

The pyrogallic acid used in this instance was a fresh supply and had a peculiar penetrating smell. Whether the failure of these cultures to grow after removal to the air is to be ascribed to the nitrogen or to some substance emanating from the pyrogallic acid must be left an open question.

Ps. campestris and *Ps. stewarti* were tested at the same time with identical results. The check tubes grew promptly. The others (2 of *Ps. campestris* and 4 of *Ps. stewarti*) made no growth whatever, either while in the nitrogen (15 days) or after being taken out (17 days). The temperature during this experiment ranged from 20° to 25° C.

(3) The third experiment did not fully accomplish what was intended, but is perhaps just as instructive. Each U tube received a tube containing 10 grams of an old stock of pyrogallic acid, not previously used, and 20 c. c. of 5 per cent caustic potash water. It browned slowly, and at the end of 48 hours a considerable part of the oxygen remained unabsorbed (perhaps one-third), and meanwhile the bacteria had begun to grow. The cultures were on coconut. Each tube was inoculated with two 3 mm. loops of *Ps. hyacinthi* from a cloudy beef-broth culture 5 days old.

Result.—The check tube grew promptly. During the first 46 hours the bacteria in the two tubes in the nitrogen (+ some oxygen) made about one-half as much growth as in the check tube. The column of mercury was now 40 mm. high. There was some additional growth in these tubes on the third day, but it was paler yellow than in the check tube. At the beginning of the fifth day the mercury stood at 58 mm. and the oxygen was probably all absorbed. From this time on there was no increase in growth. On the fifteenth day the seal was broken and the tubes removed for a more careful examination. The pale yellow growth in each tube was not more than one-twentieth as much as in the check tube.

The results were much the same in another U tube. At the end of the second day the mercury stood at 35 mm. At the beginning of the fifth day it had reached 59 mm. During this very gradual absorption of the oxygen there was some growth, but it was less than in the check tube (not over one-fifth as much), and it ceased after this date. The color of the slime in the check tube at this time was canary yellow.

The color of the slime in the tubes from the nitrogen was paler, i. e., between primrose and Naples yellow. This U tube was also opened on the fifteenth day, at which time the growth was still pale yellow and not over one-thirtieth as abundant as in the check tube.

In 8 days from the time these 4 tubes were removed from the nitrogen there was an abundant, smooth, wet-shining, bright yellow growth in each tube. This new growth began to be visible at the end of the second day. That a considerable portion of the germs were injured by exposure in the U tube was, however, shown by the fact that scrapings taken from the rather dry bacterial layer in each one of these tubes when they were first opened and put into as many tubes of beef broth failed to cloud them in 8 days.

Ps. campestris and *Ps. stewarti* were tested at the same time. *Ps. campestris* was grown on cylinders of flat white turnip in distilled water and *Ps. stewarti* on similar cylinders of sugar beet, i. e., each one on a medium specially adapted to its growth. In the check tubes growth was prompt and abundant.

In the U tube containing *Ps. campestris* the mercury had risen only 30 mm. in 46 hours, and there was nearly or quite as much growth in these tubes as in the check. On the beginning of the fourth day the mercury stood at 50 mm., and the growth was comparatively feeble, i. e., not one-twentieth as much as in the check. On the fifteenth day when the seal was broken the slime had dried away and there was no apparent growth in either tube. Eight days later each cylinder was covered with a copious pale yellow, smooth, wet-shining slime which also filled the fluid. This increased growth began to be visible the second day. A second U tube gave identical results.

In the U tube containing *Ps. stewarti* the mercury had risen only 15 mm. in 46 hours and there was about as much growth as in the check. At the beginning of the fourth day the mercury stood at 50 mm., and the growth was now not one-fifth as much as in the check tube. At the beginning of the fifth day the mercury stood at 55 mm., i. e., nearly all of the oxygen was absorbed and the growth was not one-tenth as much as in the check tube. At this time the color of the growth in the check tube was between buff yellow and deep chrome, that in the tubes in the nitrogen was "pale yellow." On the fifteenth day when the seal was broken there was not in either of these tubes over one-thirtieth as much growth as in the check, and it was paler yellow. In the fluid in the bottom of the check there was also a copious buff-yellow precipitate, but there was none in either of the tubes which had been in the nitrogen. Here, again, something seems to have done injury to the organisms, for after breaking the seal and exposing them to the air there was little increase in growth (8 days). The check was deep buff yellow. In the tubes which had

been in the nitrogen there was no buff yellow, but only a thin whitish growth. These 3 tubes were each inoculated in the same way, i. e., with 2–3 mm. loops from a beef-broth culture 5 days old.

GROWTH IN VACUO.

(1) The first test was in a partial vacuum with the remnant of the oxygen absorbed. Under the bell jar with the cultures was a beaker containing 5 grams of pyrogallic acid. In this beaker was a U tube, the short arm open, the long arm closed, and containing 30 c. c. of 13 per cent caustic potash water, with a small bubble of air at the top. The size of this bubble was so regulated that its expansion would begin to force over the potash water into the pyrogallic acid when four-fifths of the air was exhausted. The exhaustion was continued until the mercury in the cistern barometer stood at 2¼ inches. The stopcock was then turned and the apparatus separated from the pump, well sealed, and put away in the dark. The temperature during the experiment was 20° to 26° C.

Eight test-tube cultures of *Ps. hyacinthi* were subjected to this experiment. Four were on coconut (stock 395), each being inoculated with one loop of yellow slime from tube 27, February 2. Four were on potato (stock 385), each being inoculated with two loops of fluid from the bottom of tube 29, February 2, after long shaking. Two tubes of each set were placed under the bell jar and the other 4 tubes were held as checks. The experiment was begun on February 9 and the seal of the jar was broken February 18, at which time the vacuum continued as perfect as when first made.

Result.—The 4 check tubes each showed a distinct yellow growth at the end of 48 hours, and this growth continued in a typical manner. The 4 tubes in the vacuum showed no growth whatever at the end of the ninth day, when the vacuum was broken. Twenty-four hours later there was no visible growth in any of these tubes. At the end of 48 hours the 2 potato cultures showed no growth; the coconut cultures showed a slight yellow growth on the inoculated face. At the end of the third day the coconut cultures showed two or three times as much growth as at the end of 48 hours, but the growth was still thin and did not cover all of the cylinder, i. e., was not more abundant than the growth in the check tubes at the end of the third day. One of the potato cultures now showed a feeble yellow growth (less than the check tube showed at the end of the second day), and there was still no visible growth or graying of the substratum in the other tube. Six days after removal from the vacuum there was a moderately abundant bright canary yellow slime covering all that part of the coconut cylinders which projected out of the water. One of the potato cultures now contained about as much growth as the check tube, while the other also showed some growth (4 sq. cm.). In other

words, there was no growth in the oxygen-free vacuum; and 9 days' exposure to it while not killing all of the organisms probably killed many of them, since subsequent growth in the air was distinctly retarded.

Cultures of *Ps. stewarti* and of *Bacillus amylovorus* were also exposed to this vacuum. *Bacillus amylovorus* was inoculated on ordinary slant beef extract peptone agar. On this substratum, which probably contained a little muscle sugar, it made a slight but distinct growth. The check tube developed promptly, and made a good white growth the whole length of the streak. The growth in vacuo was about one-tenth to one-fifteenth as much as in the air. The 4 check tubes of *Ps. stewarti* (2 coconut, 2 potato) developed a distinct buff yellow growth within 48 hours. The 4 tubes in vacuo made no growth whatever during the 9 days' exposure, and after removal to the air growth in each one was even more distinctly retarded than in case of *Ps. hyacinthi.*

(2) The second test was made in the same manner as the first, except that the vacuum was not so complete and the remnant of oxygen was not removed. The experiment was begun March 8, and the seal was broken March 20. The mercury in the cistern barometer was down to 3 inches when the jar was sealed, and the vacuum kept quite well. The temperature during the experiment was 16° to 25° C. (mostly 20° to 22° C.).

Four organisms were tried in this jar, *Ps. hyacinthi*, *Ps. campestris*, *Ps. stewarti*, and *Bacillus carotovorus.* The media used were carrot (stock 402), alkaline beef broth (stock 382), coconut (stock 412), and potato (stock 406). All of the check tubes but one made a "feeble" to "good" growth within 48 hours, and all showed a "good growth" at the end of the third day except one tube of *Ps. hyacinthi* on potato, which lagged and was doubtful, but which 2 days later showed the typical yellow growth over about 4 sq. cm. The tubes in the vacuum were distinctly different. On the fifth day *Ps. hyacinthi* showed some growth on coconut and potato, but it was not as yellow as in the air. The same was true of *Ps. campestris* and *Ps. stewarti.* Each showed some growth, and neither was as yellow as in the checks. On the eighth day the mercury stood at $3\frac{3}{4}$ inches, and none of the potato cylinders were grayed. The condition on the twelfth day (March 20), when the seal was broken, and on subsequent days, was as follows:

Ps. hyacinthi:

(a) *Carrot.*—March 20, no visible growth (there was no check upon this tube); March 23, no growth; March 31, no growth; April 5, a smooth, wet-shining, transucent growth now covers the whole exposed surface of the carrot, and the precipitate is yellow; April 17, slime and fluid distinctly acid.

(b) *Beef broth.*—March 20, fluid clear, precipitate very slight (2 mm. broad), colorless; no rim, no pellicle, no zooglœæ; March 23, feebly clouded; March 31, well clouded.

(c) *Coconut.*—March 20, a very thin pale-yellowish growth, not one one-hundredth as much growth as in the check tube; the difference in color was not due to unlike volumes; bulk for bulk on white paper the slime from the check tube was yellower than that from the tube exposed to the vacuum. March 23, a thin growth covers 5 to 6 sq. cm.; it is yellow, but rather pale for this substratum. March 31, a bright yellow growth now covers most of the aerial portions of the cylinder.

(d) *Potato.*—March 20, a distinct but feeble pale-yellow growth; about one-tenth as much growth as in the check tube; the potato has not grayed; bulk for bulk on white paper the slime from the check tube is yellower; compared as a whole the culture in the check tube was a canary yellow; that from the vacuum was primrose yellow. March 23, a thin growth covers 2 to 3 sq. cm.; it is yellow, but seems unusually pale.

Ps. campestris:

(a) *Carrot.*—March 20, a very feeble growth; no check tube. March 23, a feeble, wet growth which does not mask the color of the carrot. March 31, a feeble growth; substratum not hidden. April 17, slime and fluid distinctly acid.

(b) *Beef broth.*—March 20, clear; a very slight white precipitate closely resembling that of *Ps. hyacinthi;* not over one-twentieth as much precipitate as in the corresponding tube of *Ps. stewarti.* March 23, feebly clouded; March 31, well clouded.

(c) *Coconut.*—March 20, a thin, pale-yellow growth over the whole aerial part of the cylinder; about one-fiftieth as much growth as in the check tube, and paler yellow; the difference in color was also apparent when equal volumes of the slime were placed side by side on white paper. March 23, 8 to 9 sq. cm. of rather pale-yellow slime. March 31, a distinctly yellow growth over the whole exposed surface.

(d) *Potato.*—March 20, the fluid is moderately cloudy and a thin, very pale-yellow growth covers the whole aerial part of the potato; there is no precipitate, no graying of the potato, no thickening of the fluid or color in it; the check culture is much yellower and contains fully 100 times as much growth; the check tube is wax yellow; the other culture is as pale as primrose yellow; side by side on white paper in equal quantities the slime of the check tube was yellower. March 23, the entire aerial part of the potato is covered with a yellow slime which also begins to fill the water; it is still rather pale but begins to recover its color and vigor. March 31, a copious typical growth.

Ps. stewarti:

(a) *Carrot.*—March 20, only the slightest trace of growth; no check tube. March 23, a slight growth, scarcely visible. March 31, fluid well clouded; out of the water there is a thin slime which does not hide the carrot. April 17, slime and fluid distinctly alkaline.

(b) *Beef broth.*—March 20, fluid very feebly clouded; a pale-yellow precipitate, 6 mm. in breadth, i. e., more than in the corresponding tubes of *Ps. hyacinthi* and *Ps. campestris;* check tube twice as cloudy and with double the precipitate, which is yellower; this organism seems to be able to get along with less oxygen than *Ps. hyacinthi* or *Ps. campestris.* March 23, fluid feebly clouded; cloudier than when taken out. March 31, well clouded.

(c) *Coconut.*—March 20, a very thin, very pale-yellow growth; the check tube contains several times as much growth and it is yellower; the one is buff yellow, the other is cream (Ridgway); removed from the tube and examined bulk for bulk and side by side on white paper, the slime from the exposed tube was also distinctly paler. March 23, a very thin, buff-yellow growth covers 4 to 5 sq. cm; it is paler than usual. March 31, there is now a thin, buff-yellow layer over the whole exposed surface.

(d) *Potato.*—March 20, a pale buff-yellow growth about one-third to one-half as abundant as in the check tube; potato not grayed, color only a little paler than

that in check tube. March 23, a very thin, pale buff-yellow growth covers 4 to 5 sq. cm; the slime is very pale yellow for the amount of growth; the potato in the air begins to gray. March 31, growth feeble.

Growth in Hydrogen.

Two tests were made in hydrogen. The gas was prepared by the action of zinc on c. p. sulphuric acid dissolved in distilled water (acid 1 part, water 9 parts). It was produced in quantity in a Kipp generator and was freed from impurities by passing it through strong solutions of argentic nitrate, potassium permanganate, and sodium hydrate. It was finally allowed to bubble through a jar of distilled water and then passed into the culture chamber. This zinc was certified to be free from arsenic and subsequent tests did not reveal any of this substance. To facilitate the removal of air, the gaseous contents of the well-luted bell jar was pumped out before allowing the hydrogen to enter. The jar was then repeatedly pumped out and refilled with the hydrogen, so that only a trace of oxygen could have remained. During the preliminary trial exhaustions, leaks were of course discovered in various places and were waxed or screwed tight. At the beginning of each experiment everything was gas tight and remained so until its close (16 days). The exposures were in a large Novy jar. At the close of each experiment the tightness of the seal was demonstrated by the fact that when the 4 clamp screws were loosened hydrogen passed out through the broad vaselined rubber joint (with a slight sound) in hundreds of tiny branching whitish rivulets and then air began to pass into the jar in the same curious way.

(1) The first experiment was begun June 14 and closed June 30. The temperature during this period was the ordinary room temperature of Washington (usually 25° to 30° C. in June). The inoculations were all into test tubes, using in case of each tube and of each organism one 2-mm. loop of cloudy beef broth 3 days old. The culture media tested were potato (stock 519), +15 beef broth (stock 473a), and +15 nutrient slant agar (stock 516), i. e., media well adapted to these organisms. Various bacteria were tested. The observations on opening the jar June 30 (sixteenth day) and on subsequent days are given below:

Ps. hyacinthi:

(a) *Potato.*—June 30, no growth. July 2, no growth; plenty of water in the tube. July 5 (end of fifth day), doubtful; there seems to be feeble clouding and a slight growth on the potato out of the water. July 9, distinct feeble, pale-yellow growth; potato grayed; fluid feebly browned. July 16, a thin, yellow, typical growth covers a portion only of the exposed potato; there is also a small amount of yellow precipitate; fluid abundant; a marked retardation of growth.

(b) *Beef broth.*—June 30, clear; no growth. July 2, clear. July 5, no growth. July 9, not cloudy; July 16, clear; no growth.

(c) *Agar.*—June 30, no growth. July 2, no growth. July 5, no growth. July 9,

no growth. July 16, no growth; the agar is still quite moist, i. e., it has dried out only a little; this tube and the two preceding were inoculated from the same culture, tube 4, June 11 (stock 473a), which was well clouded.

Ps. campestris:

(a) *Potato.*—June 30, no growth. July 2, no growth. July 5, no growth. July 9, no growth. July 16, no growth; plenty of water in the bottom of the tube and the aerial part of the potato moist; this tube and the two following were inoculated June 14 with a large 2-mm. loop from tube 13, June 11 (a beef-broth culture inoculated with a 2-mm. loop of yellow slime from a potato culture 36 days old); tube 13, June 11, clouded in 24 hours, and was well clouded in 48 hours.

(b) *Beef broth.*—June 30, clear; no trace of growth. July 2, clear. July 5, lost by accident.

(c) *Agar.*—June 30, no growth. July 2, no growth. July 5, no growth. July 16, no growth; failure is not to be accounted for by any drying out of the agar.

Ps. stewarti:

(a) *Potato.*—June 30, no growth. July 2, no growth; plenty of water in the tube. July 5, no visible growth. July 9, potato grayed, a very feeble buff-yellow growth, not over one-fiftieth as much as in the corresponding tube of *Ps. hyacinthi*. July 17, a feeble buff-yellow growth in the air; potato quite gray; fluid feebly browned; very little precipitate; a marked retardation of growth; this culture and the two following were inoculated from tube 22, June 11 (stock 473a), a well-clouded culture.

(b) *Beef broth.*—June 30, clear; no growth, or a very slight one which has settled; the nature of the slight precipitate is doubtful; it was not examined microscopically; it is possible that a trace of oxygen was left in the medium, and this organism seems to require less O. for its growth than *Ps. hyacinthi* or *Ps. campestris*. July 2, fluid feebly clouded; no rim or pellicle, but many small zooglœæ. July 5, well clouded. July 9, well clouded; no pellicle or rim. July 16, moderately cloudy, no rim or pellicle, but numerous small zooglœæ and a moderate amount of yellow precipitate; no decided retardation of growth in the air.

(c) *Agar.*—June 30, a distinct but very feeble growth; it is visible to the naked eye in the V and on the slant, if the tube is held up to the light, but it is best seen with a Zeiss × 6 aplanat; under this magnification there appear to be 300 or 400 tiny whitish colonies on the slant surface, and in the fluid a feeble clouding and some tiny zooglœæ; no yellow color is visible. July 2, a streak composed of several hundred small white colonies barely visible to the naked eye. July 5, a pale yellow growth now covers about 1 sq. cm. in the lower part of the streak. July 9, some increase of the yellow growth, but not over one-third of the slant covered; farther up there are more than 100 minute colonies, which can be seen only with a lens. July 16, growth on the lower part of the slant has doubled and is yellower than it was (buff-yellow); the tiny colonies on the middle and upper part of the slant have not increased any in size; they are dead; the agar has dried out but little.

B. pyocyaneus-pericarditidis:

(a) *Potato.*—June 30, no visible growth. July 2, an abundant growth, whitish with a tinge of yellow; no fluorescence. July 5, potato grayed throughout. July 9, a thin dirty white (or brownish white) growth; no fluorescence; growth in the air not retarded by the hydrogen.

B. amylovorus:

(a) *Potato.*—June 30, no growth. July 2, fluid well clouded and a distinct white growth on the potato out of the water. July 5, fluid heavily clouded; potato feebly grayed; growth in the air not retarded.

B. coli:

(a) *Potato.*—June 30, fluid well clouded, doubtful as to growth out of the water; if any, it is slight and of the same color as the potato; potato not grayed. July 2, scanty wet-shining white growth on the potato out of the water; organism will grow in hydrogen.

(2) The second test was begun June 16 and closed July 1. Four media were used, viz, +15 peptonized beef broth (stock 473a), peptone water with addition of grape sugar and methylene blue (stock 489), peptone water with sodium chloride and rosolic acid (stock 493), and Uschinsky's solution (stock 496). Various organisms were tested. Each tube received an equal quantity of the culture fluid, i. e., one 2 mm. loop of cloudy broth from cultures five days old. The media used had already been tested and the various organisms were known to grow well in it. The inoculations were all made from media in which the various organisms grew well, viz, peptonized beef broth neutral to phenolphthalein (stock 515e). The general management of the experiment in other particulars was the same as in the preceding.

The seal was broken, as before, on the sixteenth day (July 1), and the results were as follows:

Ps. hyacinthi:

(a) *Beef broth.*—July 1, no growth. July 2, clear. July 5, clear. July 9, clear. July 16, feebly clouded; good rolling clouds on shaking; a great retardation of growth.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; on removal the fluid was nearly colorless, but the surface layer in contact with the air immediately became greenish blue and in a few minutes the whole fluid was oxidized to this color; this result also shows that the jar remained free from oxygen. July 2, clear, July 5, clear; July 9, clear; no visible growth. July 16, well clouded; color wholly reduced, except in a thin layer at the top next to the air; this growth and reduction of color began about July 12, on shaking, the color comes back, but is again reduced on standing for a few minutes; marked retardation of growth.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; color of the fluid the same as in the uninoculated tubes. July 2, clear. July 5, no growth. July 9, clear. July 16, no growth visible; no change of color.

Ps. campestris:

(a) *Beef broth.*—Lost by breaking.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; fluid nearly colorless when taken from the jar; on contact with the air it began to color at once and in a few minutes was greenish blue. July 2, clear. July 5, clear. July 9, clear. July 16, fluid greenish blue; no growth.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; the fluid is the same color as when inoculated. July 2, clear. July 5, no growth. July 9, clear. July 16, clear; no change in color.

Ps. stewarti:

(a) *Beef broth.*—July 1, no growth. July 2 (temperature 28° C.), very feebly clouded. July 5, moderate clouding, most in the upper 6 mm. where there are numerous small zoogloæ which stream down on gentle shaking; much increase in growth since July 2, but no rim or pellicle; July 9, moderately cloudy; no rim, but a delicate pseudo-pellicle of separate zoogloæ. July 16, feebly clouded.; a moderate amount of yellow precipitate and a thin fragile yellow iridescent pellicle, which breaks up on slight shaking into a great many roundish zoogloæ; growth in the air not distinctly retarded.

(b) *Grape sugar peptone water with methylene blue.*—July 1, clear. There seems to have been a little growth—i. e., there are a few tiny floating flecks of uncertain nature, there is a small amount of colorless precipitate which is wanting in the cor-

responding tubes of *Ps. hyacinthi* and *Ps. campestris*, and the reduced fluid oxidizes back on contact with the air to a color which is bluer than that in the tubes already mentioned, and which resembles that in a tube of Jones's carrot rot organism (*Bacillus carotovorus*), where there has certainly been some growth. July 2, feebly clouded; rolling clouds on shaking. July 5, feebly clouded. July 9, clear or very nearly so; no rim or pellicle. July 16, clear; no reduction; fluid a pure blue; a feeble growth after removal to the air, but no marked retardation.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; fluid the same color as when inoculated. July 2, clear. July 5, there seems to be a slight deepening of the color, the clouding is not distinct. July 9, not much change. July 16, as on the 9th; fluid slightly pinker than in the corresponding tubes of *Ps. hyacinthi* and *Ps. campestris*; not cloudy.

(d) *Uschinsky's solution.*—July 1, no growth. July 2, clear. July 5, clear. July 9, clear. July 16, clear; no growth.

B. pyocyaneus pericarditidis:

(a) *Beef broth.*—July 1, a slight growth, which has not increased any of late; the fluid is clear and there is no rim, but there is a small amount of precipitate (10 mm. wide), and a bacterial film invisible to the naked eye, but distinct under the lens, and covering about one-sixth of the surface. July 2, moderately cloudy; fluid green fluorescent in the upper one-fourth and bearing a thick white pellicle. July 5, very heavy clouding and a marked increase of the fluorescence; an abundant white precipitate, a thin white rim, and a white pellicle which settles easily on jarring. July 9, very cloudy, but fluorescence not pronounced. July 16, fluid well clouded; very ropy; only slightly fluorescent, feebly browned; precipitate 4 mm. deep; no retardation of growth after removal to the air.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth or only the merest trace; fluid nearly colorless; it becomes greenish in a few minutes on exposure to the air. July 2, fluid clouded, with a thin pellicle; color half reduced. July 5, fluid well clouded and uniformly blue, if any reduction of color it is uniform; some of the pellicle has fallen; under the fluid there is a thin white rim 1½ mm. wide. July 9, cloudy blue with a thin white rim and pellicle. July 16, the pellicle has fallen; the fluid is pure blue; there is no distinct fluorescence or reduction of color.

(c) *Salted peptone water with rosolic acid.*—July 1, a trace of growth; not cloudy, but with a slight precipitate and a membranaceous pellicle visible only under a lens; no rim. July 2, fluid clouded, pellicle more distinct; the color has turned toward pink. July 5, moderately cloudy; no pellicle, that of July 2 lies on the bottom unbroken; there is a thin rim under the surface of the fluid; the latter is now bright pink; it was originally yellowish rosaceous and the uninoculated tubes are still that color. July 9, much as on 5th. July 16, fluid moderately cloudy, color bright pink red.

B. amylovorus:

(a) *Beef broth.*—July 1, clear; no present growth; there is a slight precipitate, chemical (?); it is much less than in case of *B. coli*. July 2, clear. July 5, clear. July 9, clear. July 16, clear; no growth, unless possibly when first placed in the hydrogen.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; fluid nearly colorless when removed, but soon changing to a greenish blue, as in case of *Ps. hyacinthi* and *Ps. campestris*. July 2, clear. July 5, clear. July 9, clear. July 16, no reduction; no clouding; fluid "pure blue."

(c) *Salted peptone water with rosolic acid.*—July 1, no rim, pellicle, or clouding. A slight rosy precipitate (2 mm. wide), which is possibly chemical; the color of the fluid is the same as when inoculated. July 2, clear. July 5, no growth; no change in color. July 9, as on the 5th. July 16, as on the 5th.

B. coli:

(a) *Beef broth.*—July 1, some growth; no rim or pellicle; only the merest trace of clouding and no rolling clouds on shaking, but a white precipitate 10 mm. broad. July 2, well clouded; a thin white rim, and a gathering of zoogloeæ into the upper layers which are cloudiest. July 5, heavily clouded, more so than on the 2d; a thin white pellicle and a white rim 3 mm. wide. July 9, as on the 5th; the pellicle settles on very gentle shaking.

(b) *Grape sugar peptone water with methylene blue.*—July 1, a slight growth; fluid feebly clouded; no rim, but some slight fragments of pellicle and a precipitate 4 mm. wide; fluid nearly colorless; on exposure to the air the fluid becomes *bluish*, i. e., like the carrot-rot culture; the uninoculated tubes are greenish. July 2, heavily clouded; there has been no reduction of the color; it is now a pure bright blue (brighter than yesterday). July 5, well clouded; no rim or pellicle; fluid (by transmitted light) a uniform bright blue. July 9, as on the 5th. July 16, fluid pure blue, no reduction of color; moderately cloudy, no rim, no pellicle; a scanty bacterial precipitate which is *blue.*

(c) *Salted peptone water with rosolic acid.*—July 1, a rosy precipitate 3 mm. wide; no clouding, no rim, no pellicle. July 2, moderately cloudy; fluid is changing to pink. July 5, well clouded; no rim or pellicle; fluid deep pink; at least twice as much color as in the corresponding tube of *B. pyocyaneus pericarditidis.* July 9, as on 5th. July 16, feebly clouded, slight precipitate; no rim or pellicle; fluid deeper red than that in the corresponding tube of *B. pyocyaneus-pericarditidis.*

Growth in Carbon Dioxide.

The carbon dioxide was prepared in quantity in a Kipp generator from boiled marble chips and c. p. hydrochloric acid diluted with distilled water (1 part acid, 9 parts water). The gas was allowed to flow until all air was displaced from the apparatus. It was washed in 1 per cent caustic potash water and then in distilled water. The tubes were exposed in a deep specimen jar with a flat brass top provided with inflow and outflow tubes having very perfect stopcocks. When all was ready a waxed rubber gasket was laid on the top of the jar and the solid brass top was clamped down securely. The jar was first exhausted of air until the mercury stood at 3 inches. It was then filled with the CO_2 five times, and as many times pumped out. After the sixth filling the stopcock was turned off and everything sealed securely. Preliminary exhaustion tests had shown only a slight leakage, i. e., in 24 hours the mercury in the cistern barometer rose only from 2½ to 3½ inches.

The following media were tested: Tubes of beef broth neutral to phenolphthalein (stock 382); tubes of potato (stock 405); tubes of coconut (stock 412); slant beef-extract peptone agar neutral to litmus. Each tube was inoculated copiously and in the same way, i. e., with large loops from well-clouded beef broth cultures 13 days old. Two or more tubes of each medium were inoculated and one of each medium was held as a check. The exposure was begun March 10 and the tubes were removed to the air after 10 days, i. e., on March 20. On taking off the brass cover, lighted matches were repeatedly plunged into the

jar and as often extinguished. They went out instantly they were depressed below the level of the top of the jar. The checks behaved well. On the sixth day in each one there was a well-developed typical growth of the particular organism used.

The results obtained in the CO_2 and on continuing the cultures in the air are stated below. The temperature during the experiment did not vary much from 22° C.

Ps. hyacinthi:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, moderately cloudy with rolling clouds on shaking; a slight precipitate. March 31, well clouded; growth retarded by the CO_2.

(b) *Potato.*—March 20, no growth. March 21, no growth. March 23, no growth. March 27, a typical growth, wet and distinctly yellow on the lower one-half of the exposed part of the potato; that part out of the water is graying. March 31, the yellow slime now covers all of the potato out of the water.

(c) *Potato.*—March 20, no growth. March 21, no growth. March 23, no growth. March 27, same appearance as in the preceding; growth retarded by the CO_2. March 31, like the preceding.

(d) *Coconut.*—March 20, no growth. March 21, a thin yellow growth now covers about 1 sq. cm.; growth in the air not retarded by the exposure. March 23, a thin distinct yellow growth now covers 6 to 7 sq. cm. March 27, 9 sq. cm. of bright yellow growth.

(e) *Coconut.*—March 20, no growth. March 21, a thin, yellow growth now covers about 3 sq. cm. March 23, like the preceding. March 27, like the preceding.

Ps. campestris:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, no growth. March 31, no growth; fluid still alkaline; it was now reinoculated with a small amount of yellow slime from potato culture *b*, and on April 5 was well clouded with a yellow rim and numerous zoogloœæ; exposure to CO_2 appears to have destroyed the organism.

(b) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble pale-yellow growth now covers part of the potato; growth retarded by the CO_2. March 27, a copious wet-looking, distinctly yellow slime on the exposed parts of the potato and in the fluid.

(c) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, resembles the preceding—less growth but more color; growth retarded. March 27, like the preceding.

(d) *Coconut.*—March 20, no growth. March 21, no visible growth. March 23, a thin yellow growth now covers 5 sq. cm. March 27, a yellow growth now covers nearly all the cylinder out of the water; no distinct retardation.

(e) *Coconut.*—March 20, no growth. March 21, no visible growth. March 23, a thin yellow growth covers 3 sq. cm. March 27, like the preceding.

Ps. stewarti:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, no growth. March 31, no growth; reinoculated with a small amount of slime from the coconut culture *d*. On April 5 the culture was well clouded and had a good rim; exposure to the CO_2 appears to have destroyed the organism.

(b) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble, patchy, buff-yellow growth now covers 6 sq. cm. March 27, a typical buff-yellow growth; marked graying of the potato in the air.

(c) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble, patchy, buff-yellow growth covers 3 sq. cm.; neither potato has grayed. March 27, like the preceding.

(d) *Coconut.*—March 20, no growth. March 21, extremely thin (barely visible) buff-yellow growth over 3 sq. cm. March 23, a thin, pale, buff-yellow growth now covers about 5 sq. cm. March 27, a rather scant buff-yellow growth over 8 sq. cm. No retardation.

B. amylovorus:

(a) *Agar.*—March 20, no growth. March 21, a distinct growth on the lower end of the slant. March 23, the white growth slowly increases.

This closes my studies of the aerobism of *Ps. hyacinthi* and related species. All the various experiments lead to substantially the same conclusions: (1) *Ps. hyacinthi* and the other yellow species of Pseudomonas are more strictly aerobic than most species of bacteria; (2) while somewhat variable among themselves none of these yellow-plant parasites will survive exclusion of oxygen for more than a very few weeks; (3) nitrogen, hydrogen, and carbon dioxide seem to be only negatively harmful; (4) the organisms were more tolerant of these gases on some media than in others. They were especially susceptible in beef broth, in peptone water, and on agar.

RELATIVE NUTRIENT VALUE OF CARBON COMPOUNDS.

Bouillon and Peptone Water with Various Sugars, etc.

The few results obtained may be summed up as follows:

(1) A feeble clouding was obtained with *Ps. hyacinthi* in a fluid consisting of 1 part of strongly alkaline beef broth (286b) in 500 parts of distilled water. *Ps. campestris* and *Ps. phaseoli* also clouded this fluid. These cultures were made in clean tubes of resistant glass.

(2) *Ps. hyacinthi* grew readily in distilled water containing 1 to 2 per cent of Witte's peptonum siccum, and the precipitate was yellow. Growth in 1 per cent peptone water in the open end of fermentation tubes, as we have already seen, was increased by the addition of 1 per cent doses of grape sugar, fruit sugar, cane sugar, or dextrin, and was not perceptibly increased by the addition of 1 per cent doses of milk sugar, maltose, mannitol, or glycerol. If under these conditions any acid was formed from any of these substances, it was overlooked or obscured by the alkali.

(3) In distilled water (10 c. c. portions in tubes of resistant glass) containing 4 per cent of Witte's peptonum siccum and 4 per cent of dextrin there was little or no retardation of growth. On the twelfth day the fluid was plainly alkaline to litmus. On the twenty-ninth day there was an abundant yellow rim and a very copious dull-yellow precipitate (6 mm. deep). The cloudy fluid was plainly and rather strongly alkaline. On this date there was several times as much precipitate as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. On the fortieth day the fluid was strongly alkaline. It was still cloudy with rolling clouds on shaking, and there was no brown stain in it. On the sixty-fifth day the fluid was moderately alkaline. No crystals

were present, and a feeble brown stain, thought to have been detected on the fifty-fourth day, was not well enough developed to be recorded as certainly present. On this date there was more than twice as much precipitate as in the corresponding tube of *Ps. campestris*. This dextrin had been ten times precipitated with alcohol in the Division of Chemistry, United States Department of Agriculture. It gave a heavy yellowish precipitate on boiling 1 minute in Soxhlet's solution, but no precipitate on boiling 2 minutes in Barfoed's reagent.

(4) In distilled water (10 c. c. in tubes of resistant glass) containing 4 per cent of Witte's peptonum siccum and 4 per cent of maltose there was no retardation of growth, and for the first week or so the culture closely resembled the preceding. On the twelfth day the fluid was distinctly alkaline to litmus, but it was less cloudy than the preceding and there was far less precipitate. On the twenty-ninth day the fluid was plainly and rather strongly alkaline, but there was only about one-tenth as much precipitate as in the tube containing the dextrin. On the fortieth day the fluid was still cloudy, but was not browned. The rim was not very abundant and was paler than in the preceding. The precipitate was the same shade of pale yellow as in the tube containing the dextrin, but there was only one-tenth to one-fifteenth as much. On the sixty-fifth day the fluid was strongly alkaline, but both in this and in the preceding the blue color soon disappeared from the neutral litmus paper, leaving it redder than before. No crystals were formed.

In the corresponding tube of *Ps. campestris* there was a distinct browning of the fluid, which was first noticed on the fortieth day. *Ps. phaseoli* browned neither fluid.

Crude Vegetable Substances.

The behavior of *Ps. hyacinthi* in contact with steam sterilized solids and fluids derived from plants has been discussed so fully under Sensitiveness to acids and Growth on solid media that it is only necessary here to recapitulate a few of the more important discoveries.

(1) All my observations tend to show that plant acids, even in comparatively small doses, prevent growth, and that still smaller quantities retard growth. It is, therefore, probable that these acids do not serve directly as food. Certainly the behavior of this organism in nutrient fluids containing malic acid is extremely unlike that of organisms which are believed to use this acid as a food, e. g. *Bacillus amylovorus*.

(2) Starch, as we have seen, is transformed into substances which can be assimilated only with the greatest difficulty.

(3) Growth on steamed vegetables poor in sugar was always rather meager. Substrata containing rather more sugar gave a correspondingly better growth.

(4) Growth on vegetables rich in grape sugar or cane sugar was copious and long continued. These two sugars are excellent foods, and when not present in such excess as to inhibit growth (probably by plasmolysis) they greatly favor the multiplication of this organism.

Sugar Gelatin.

(See Growth on solid media.)

Sugar Agars.

Some interesting results were obtained by adding large doses of sugar to 10 c. c. portions of Mr. Dorsett's +15.5 meat-extract peptone agar (see Growth on solid media) and growing on it the various yellow organism in slant cultures. Their behavior on these media was always compared with that on check tubes of the sugar-free agar.

NINE PER CENT SUGARS.

First series.

Agar recently tubed and slanted (10 cc. to 1 gram of the sugar). Inoculations with *Ps. hyacinthi*, using bright-yellow slime from a starch jelly culture 28 days old. All the inoculations were made in the same way and with approximately the same amount of material.

Third day.

(1) *Check.*—Streak 2 by 75 mm., distinct the whole length of the track, best developed at the lower end, where it is distinctly pale yellow. In the middle 3 c. m. it consists of separate colonies.

(2) *Grape sugar (1 gram of Merck's c. p. anhydrous).*—Streak invisible except in a very favorable light, where it looks like a colorless film.

(3) *Cane sugar (1 gram of white commercial).*—A thin pale yellow growth over the whole slant. In strong contrast with the grape-sugar agar. Also more growth than in the check tube.

Seventh day.

(1) *Check.*—The streak is now 3 to 5 mm. wide. All of the colonies have fused into a smooth, yellow, wet-shining homogeneous surface.

(2) *Grape sugar.*—Growth mostly in the form of separate colonies and less than in the check tube—i. e., a distinct retardation. There are many of these colonies, and where they have coalesced the color is about the same shade of yellow as in the check tube.

(3) *Cane sugar.*—The whole surface of the slant agar is covered and hidden by a copious pale yellow growth. Six times as much growth as in the check tube and 8 or 10 times as much as on the grape-sugar agar.

Sixteenth day.

(1) *Check.*—The streak has not widened any. It is smooth, translucent, wet-shining, and distinctly pale yellow. The margins of the streak are thin but distinct. A penholder is plainly visible under the streak.

(2) *Grape sugar.*—Growth has quadrupled and is now about 3 times as abundant as in the check tube, but its surface is very unlike that of the latter. The surface,

which is pale yellow, has a peculiar roughened or areolate appearance, which appears to be due to wrinkles extending in various directions. The shallow pits are 2 to 3 mm. in diameter.

(3) *Cane sugar.*—Fully 6 times as much growth as in the check tube. The penholder can not be seen under it. Color pale yellow, a little paler than in the check tube. Surface not smooth as in the check tube nor wrinkled as on the grape-sugar agar, but finely roughened.

Twenty-ninth day.

(1) *Check.*—Little change. The streak is 3 to 6 mm. wide. Its surface is smooth and wet-shining, and to either side, on the lower part of the slant, there is a slight chemical whitening of the surface of the agar. No brown stain.

(2) *Grape sugar.*—About 4 times as much growth as in the check tube. The bacterial layer covers all but the upper part of the slant and there is some growth between the agar and the walls of the tube. Growth the same shade of yellow as in the check tube, or only a trifle paler. No brown stain. No whitish chemical film on the agar beyond the streak. Surface wet, but not smooth as in the check tube. The extreme upper part of the streak is still composed of separate colonies, and the rest of it is areolated i. e., covered with tiny ridges and depressions.

(3) *Cane sugar.*—Color uniformly pale yellow. Surface drier than it was and slightly roughened, but not coarsely areolate, as on the grape sugar agar. Streak less translucent than in the check tube, i. e. almost opaque. No brown stain. The culture has a feeble smell. On boiling the contents of this tube for one minute in Soxhlet's solution there was a very heavy precipitate of copper oxide. Sugar and agar had both been tested for reducing substances previous to inoculation and neither one gave any trace of copper oxide on boiling two minutes in Soxhlet. The slime remaining in the tube was very feebly alkaline to litmus, i. e., much less alkaline than might have been expected from the amount of growth. This is presumptive evidence that most of the alkali had been neutralized by some acid.

Forty-seventh day.

(1) *Check.*—The streak is drying out. It is 65 by 3 to 6 mm., i. e., it has spread but little. It is still smooth, wet-shining, and so translucent that a penholder can be seen through it. The streak has well-defined margins, beyond which the surface is feebly whitened. On neutral litmus paper the saffron-yellow slime has an alkaline reaction. Examined microscopically, this slime consists of zoogloœ and short, slender rods, single or in pairs. Rods in fours are rare, and chains are short and exceedingly rare.

(2) *Grape sugar.*—The bacterial layer is gallstone yellow. It now covers almost the entire slant (70 by 16 mm.), and is about 20 times as abundant as in the check tube. It scrapes off easily and gives an acid reaction on neutral litmus paper. A few separate colonies persist on the upper dried-out part. The surface is not smooth, but roughened, and wrinkled slightly in the lower part of the slant. Examined microscopically, the slime consists of zoogloœ, chains, and short, slender rods, single, in pairs, or in fours. Chains of 10 to 20 or more segments are numerous. In some the individual segments are easily discernible, in others not. Apparently some of the rods are motile. No spores.

(3) *Cane sugar (another tube of the same age, but containing only 6.75 per cent of sugar).*—Growth dense and finely roughened (fine wrinkles under the hand lens). No brown stain. No crystals. No chemical film. At least 10 times as much growth as in a check tube. Slime, buff yellow (R. VI-19), acid to neutral litmus paper. Examined microscopically, the slime consists of zoogloœ, numerous chains of 10 to 40 segments, and many single rods, pairs, and fours joined end to end. In many of

the chains, but not all, the individual elements are visible. No spores. The microscopic appearance closely resembles that of the slime from the grape-sugar agar, the principal difference being the tendency to longer chains or filaments.

Second series.

The check tube had the driest surface; the surface of the fruit-sugar agar was the moistest. Inoculations from a slant-agar culture of *Ps. hyacinthi* 13 days old. All made in the same way and with approximately the same amount of material.

Third day.

(1) *Check.*—Streak 78 by 5 to 12 mm., pale yellow, translucent, smooth, wet-shining, homogeneous looking, and not scanty, i. e., a good growth over the whole length of the slant.

(2) *Fruit sugar* (*1 gram of Schering's diabetine*).—No growth, although inoculated just as copiously.

(3) *Grape sugar* (*1 gram of Merck's c. p. anhydrous*).—A feeble growth consisting of scattered colonies which, in some places, have fused into a very thin layer. Not one-twenty-fifth as much growth as in the check tube. Grape sugar in 9 per cent doses distinctly retards growth. (This growth doubled during the next 24 hours.)

Fifth day.

(1) *Check.*—Much as before.

(2) *Fruit sugar.*—No growth.

(3) *Grape sugar.*—There is now nearly as much growth as in the check tube. The lower one-half of the slant is covered, and the upper one-half bears scattering yellow colonies. The surface is not smooth, as in the check tube, but is distinctly shagreened to the naked eye. The yellow slime is very feebly alkaline, inducing only the barest trace of blue on wet or dry neutral litmus paper.

Eighteenth day.

(1) *Check.*—A thin, smooth, moist, pale-yellow slime covers nearly the entire slant. There is no brown stain in the agar.

(2) *Fruit sugar.*—No growth. Fragments of the moist agar pressed on neutral litmus paper redden it.

(3) *Grape sugar.*—A copious, pale-yellow, coarsely wrinkled growth now covers the whole slant. This layer scrapes off easily, and is very feebly alkaline to neutral litmus paper. There is no brown stain in the agar.

Fifty-third day.

(1) *Check.*—Slime feebly alkaline.

(2) *Fruit sugar.*—No growth. Failure to grow was attributed to the restraining influence of lactic acid put into this sugar by the manufacturers to improve its keeping qualities.

(3) *Grape sugar.*—Streak somewhat wrinkled and on the margins slightly areolate. Slime now distinctly acid to neutral litmus paper, no trace of any alkaline reaction. Culture diluted (shaken) with 40 c. c. of distilled water and retested. It is now neutral or only very feebly acid. On boiling this water a little acid is given off in the first vapors (CO_2?), but less than from a corresponding culture of *Ps. campestris*. On concentrating this fluid by continued boiling it became plainly more acid to litmus paper, indicating the presence of a small amount of some non-volatile acid. Cultures of *Ps. campestris* behaved in the same way.

The fructose (Schering's diabetine) was first titrated with caustic soda and litmus to determine its acidity. This was such that 10 c. c. of $\frac{N}{10}$ NaOH were required to render 10 grams moderately alkaline to litmus. One-half c. c. of this thick alkaline sirup was then pipetted into 7 c. c. of Dorsett's agar for one experiment and 1 c. c. of the sirup into 10 c. c. of the agar for another experiment. The agar was then resterilized and slanted in the usual way. The check tubes had been slanted longer than the others and their surface was somewhat dry. All were inoculated with *Ps. hyacinthi* from a slant agar culture 24 days old, in the same way and with approximately the same amount of material.

Second day.

(1) *Check.*—A feeble growth in the form of scattered colonies.

(2) *Fruit sugar (one-half c. c. sirup).*—A feeble growth, which was visible sooner than in the check tube, i. e., within 18 hours.

(3) *Fruit sugar (1 c. c. sirup).*—A very slight growth, not one-fourth as much as in the preceding.

Fourth day.

(1) *Check.*—Not a good growth. It occurs colony-wise over the streak. This agar had been slanted a long time and the surface was becoming too dry for good growth.

(2) *Fruit sugar (one-half c. c. sirup).*—A distinct multiplication during the last 48 hours, but not yet a homogeneous streak, i. e., growth thin in some places and more abundant in others. Not yet more growth than would have appeared in the same time on a freshly slanted check tube.

(3) *Fruit sugar (1 c. c. sirup).*—Very little growth, i. e., not one-twentieth as much as in the preceding. This substratum evidently retards growth.

Seventh day.

(1) *Check.*—A much better growth. The colonies touch or nearly touch, forming a thin, distinctly yellow slime over nearly the whole slant.

(2) *Fruit sugar (one-half c. c. sirup).*—There is now more growth than the agar alone would give. The streak is dense and rather abundant (47 by 10 mm.), pale yellow, smooth, and wet-shining.

(3) *Fruit sugar (1 c. c. sirup).*—Growth very feeble. There has been a slight increase during the last 3 days, but the growth is not now one one-hundredth, perhaps not one one-hundred-and-fiftieth, as much as in the preceding tube.

Twelfth day.

(1) *Check.*—The colonies, for the most part, have now fused into a smooth surface.

(2) *Fruit sugar (one-half c. c. sirup).*—A very copious, pale yellow, smooth, wet-shining growth covers the whole slant, and is growing in between the tube and the agar. At least 4 times as much growth as in the check tube.

(3) *Fruit sugar (1 c. c. sirup).*—The restraining influence is being overcome. About one-third as much growth as in the preceding, and excellent where it has obtained a foothold. This growth is of the same character as in the preceding.

Sixteenth day.

(1) *Check.*—Growth decidedly yellow, still thin.

(2) *Fruit sugar (one-half c. c. sirup).*—Growth has continued. It is wet-shining, very smooth, and extremely copious. About 10 times as much growth as in the check tube. Fructose distinctly favors growth unless all of this excess is attributable to the sodium lactate formed by neutralizing the lactic acid, which is extremely improbable.

(3) *Fruit sugar (1 c. c. sirup).*—A marked increase of growth during the last 4 days. A considerable part of the slant which was then free is now covered. The slime is pale yellow; the surface is very smooth and wet-shining.

Thirtieth day.

(1) *Check.*—Surface so dry that not all of the colonies have fused. No crystals. No stain of the agar.

(2) *Fruit sugar (one-half c. c. sirup).*—The pale yellow, wet-shining, smooth slime is 3 mm. deep over the whole surface of the slant. The color is dull yellow, but there is no reason for thinking it contaminated. No brown stain. No crystals in the agar. Growth has been enormously stimulated by this sugar.

(3) *Fruit sugar (1 c. c. sirup).*—The entire surface of the slant (15 by 53 mm.) is now covered with a pale yellow, smooth, very wet-shining slime. There is no brown stain, and there are no crystals in the agar.

SEVENTEEN PER CENT SUGARS.

Mr. Dorsett's +15.5 sugar-free agar was also the basis of all of these tests. Each tube contained exactly 10 c. c. of agar to which was added 2 grams of the sugar to be tested. The slant surfaces were all inoculated in the same manner, and with approximately the same amount of material, viz, loops of bright-yellow slime from a coconut culture 8 days old.

First day (22 hours at 27° to 30° C.).

(1) *Check.*—A distinct, wide, pale yellow streak.

(2) *Grape sugar (2 gr. Merck's c. p. anhydrous).*—Streak not visible.

(3) *Cane sugar (2 gr. white commercial).*—A meager growth. One-tenth to one-twentieth as much as in the check tube. For the most part, the streak is invisible and nowhere shows more than a trace of growth.

Fourth day (temp. 27° to 31°).

(1) *Check.*—Streak smooth, wet-shining and rather bright yellow, but not dense enough to be opaque. It is 72 by 5 to 6 mm. The margins of the streak are distinct and there is no whitish efflorescence on the surface of the agar around the streak.

(2) *Grape sugar.*—Doubtful. No visible growth except in very favorable lights. If any growth at all, not one one-hundredth as much as in the check tube. There can be no doubt that 17 per cent grape-sugar agar exerts a very distinct retarding influence on *Ps. hyacinthi.*

(3) *Cane sugar.*—A well-developed streak 62 by 5 to 8 mm. It appears to be as dense as in the check tube, but is paler yellow, i. e., the color is exactly that of a 4 days' growth of *Ps. campestris* on the check agar. This tube and the check tube are in marked contrast with the preceding.

Eighth day.

(1) *Check.*—The streak has thickened a little, but has not widened.

(2) *Grape sugar.*—What looked on the fourth day like mere dried-out portions of the slime used in making the inoculation has now developed as a distinct growth in

two places, aggregating 2 square cm. Where the organism has secured a foothold the slime is distinctly pale yellow, but much of the part which was streaked bears no growth whatever, and altogether there is not one-tenth as much growth as in the check tube. The surface of this slime is not smooth, as in the check tube, but is minutely fissured and roughened all over.

(3) *Cane sugar.*—As much growth as in the check tube, but paler yellow. Thus far the sugar has not stimulated growth. The streak is now 5 to 9 mm. wide. It has thickened some since the last record, but has not widened much.

Thirteenth day.

(1) *Check.*—Streak well developed, smooth, wet-shining, and distinctly yellow. The margins are well defined, and the body of the streak is not opaque, i. e., the penholder can still be seen through it. It shows very little tendency to spread, i. e., it is still only 5 to 7 mm. wide. There are no projections from the under side of the streak into the agar (such growths appeared in case of an undescribed, white, spore-bearing organism, derived from rotting tomato fruits, and grown on this same agar). There is now a slight but distinct bloom (chemical whitening) on the surface of the agar beyond the streak.

(2) *Grape sugar.*—About one-third, or possibly one-half as much growth as in the check tube. The color is the same, but the surface appearance is very different. The wet surface is not smooth, but is roughened, or areolated, as if made up of fused zooglœæ with grooves between them. There is no chemical whitening of the surface of the agar beyond the streak.

(3) *Cane sugar.*—Streak mostly 6 to 10 mm. wide. Surface drier and paler yellow than in the preceding or in the check tube. No growths into the agar from the under surface of the streak. Seventeen per cent cane-sugar agar is not nearly so favorable to the growth of this organism as 9 per cent. There is now but little more growth than in the check tube, whereas on the 9 per cent cane-sugar agar there was 6 times as much growth in one-half this time, the temperature in both cases being approximately the same, i. e., near the optimum.

Seventeenth day.

(1) *Check.*—The yellow slime is plainly alkaline to good neutral litmus paper, much more so than that on the grape-sugar or cane-sugar agar.

(2) *Grape sugar.*—Slime neutral or very slightly alkaline.

(3) *Cane sugar.*—Slime neutral or only very slightly alkaline.

Thirtieth day.

(1) *Check.*—The streak begins to dry out. Its surface is smooth. There has been no widening. Beyond the streak the whitish chemical film remains, but is not very pronounced. No brown stain. No crystals.

(2) *Grape sugar.*—A pale yellow well-developed streak (50 by 5 to 9 mm.). It has not spread widely, and is still translucent. The surface is rather coarsely roughened and looks as if many large zooglœæ had fused, leaving grooves between each one. The surface of the individual hummocks is smooth, wet-shining, firm, elastic, and scrapes off only after the use of considerable force. Examined under the microscope, this growth consists of slender rods mixed in with some chains. The rods are single, in pairs, and in fours joined end to end. There is no brown stain.

(3) *Cane sugar.*—The growth is now two or three times as abundant as in the check tube. It is very dense, especially on the lower part of the slant, where it is crowded up into high folds. The upper part shows lesser wrinkles. No brown stain and no chemical film on the clear agar to either side of the streak. The slime is pale yellow

and very feebly alkaline. It is made up of small roundish zoogloeæ, short chains of a dozen or more segments, and slender short rods, single, in pairs, or fours. Some of the rods are actively motile.

Sixty-sixth day.

(1) *Check.*—Slime strongly alkaline to neutral litmus.
(2) *Grape sugar.*—Slime not alkaline. Distinctly acid on neutral litmus paper.
(3) *Cane sugar.*—No alkaline reaction. Slime distinctly acid on neutral litmus paper.

TWENTY-THREE PER CENT SUGARS.[1]

These cultures were like the preceding except that for each 10 c. c. of agar 3 grams of the specified sugar was used. The check tubes had been slanted longer than the others and their surface was drier. All were smeared with *Ps. hyacinthi* from an agar culture 24 days old in the same way and with approximately the same amount of material. The alkaline fruit-sugar agars already described were inoculated at the same time and from this same culture, which was the check tube described under the 17 per cent sugar agars.

Seventh day.

(1) *Check.*—A thin distinctly yellow growth over nearly the whole slant.
(2) *Grape sugar (3 grams Merck's c. p. anhydrous).*—No growth.
(3) *Cane sugar (3 grams white commercial).*—A very feeble, scrappy growth, not forming a streak, but confined to the immediate vicinity of some small fragments of slime, which were left unspread when the agar was inoculated. Not more than two or three times as much slime present as was put into the tube in making the inoculation. Twenty-three per cent cane-sugar agar strongly retards growth.

Twelfth day.

(1) *Check.*—Fully twice as much growth as on the cane-sugar agar.
(2) *Grape sugar.*—No growth, although the surface of the entire slant was rubbed with a mass of yellow slime as large as a pin head.
(3) *Cane sugar.*—A distinct, rather thin, wet, yellow, rough-surfaced growth, which covers about one-half of the slant (lower half).

Thirtieth day.

(1) *Check.*—Surface of the streak smooth, wet-shining, and distinctly yellow; no reticulations or shagreen.
(2) *Grape sugar.*—No growth. *Ps. hyacinthi* will not grow on 23 per cent grape-sugar agar.
(3) *Cane sugar.*—The lower three-fourths of the slant is covered with a distinctly yellow growth, which is rather dry, but looks wet under the hand lens. The surface is not smooth, but is reticulate, areolate, or shagreened, the portions between the grooves being lighter yellow and very smooth. This areolation is shown in Bulletin 26 of this Division, in text fig. 3, which was made from this culture on the thirty-third day. The agar has not dried out much, but the slime shows no tendency to flow.

[1] The expressions 9,17 and 23 per cent are used for convenience. Of course, the writer is aware that 3 grams added to 10 c. c. does not make exactly 23 per cent.

Thirty-seventh day.

(1) *Check.*—No record.

(2) *Grape sugar.*—No record.

(3) *Cane sugar.*—No crystals. No stain in the agar. The bacterial layer peels off easily in fragments, leaving a smooth, clean agar surface. This layer is not sticky or elastic, and dissolves with difficulty in water, breaking up into rather coarse fragments. Examined under the microscope, it consists of zoogloœ, single rods, doublets, and chains. The latter are 50 to 100 μ. long.

SUMMARY OF RESULTS WITH SUGAR AGARS.

(1) *Ps. hyacinthi* grew without retardation on the check tubes, and the surface was always smooth.

(2) Addition of 9 per cent grape sugar retarded growth. Finally growth was more abundant than in the check tubes, and the surface was areolated.

(3) Addition of 17 per cent grape sugar retarded growth for a longer time. This was finally more abundant than in the check tubes, and its surface was areolated.

(4) Addition of 23 per cent grape sugar entirely prevented growth.

(5) Addition of 9 per cent cane sugar did not retard growth, and after a few days greatly stimulated it. The surface was wrinkled or finely roughened.

(6) Addition of 17 per cent cane sugar retarded growth. This finally became more copious than in the check tube, but it was never as abundant as on the 9 per cent cane-sugar agar. The surface was wrinkled.

(7) Addition of 23 per cent cane-sugar agar retarded growth for a longer time, but did not prevent it. The surface was areolated or shagreened.

(8) Addition of 9 per cent acid fructose (Schering's diabetine) entirely prevented growth. When the lactic acid was neutralized by caustic soda growth ensued, but was retarded for some time. In the end it was very abundant.

Some interesting comparisons were obtained from concomitant cultures of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*.

(1) On the check or sugar-free agar all three grew without retardation, and did as well as *Ps. hyacinthi*. This agar was not stained brown and no crystals were formed, but the superficial white chemical film appeared whichever organism was used. This film also failed to appear on the sugar agars, whichever germ was used. In the check tubes of each the slime was feebly alkaline at first and finally became strongly alkaline. On the contrary, with grape sugar or cane sugar, the reaction of the slime changed very slowly from alkaline to acid, whichever organism was used. All four invert cane sugar. All are alike in producing a small amount of non-volatile acid when grown on

this agar in the presence of grape sugar or cane sugar. All were much alike in color, but frequently the hyacinth germ was the brighter yellow.

(2) The growth of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti* was not retarded by 9 per cent grape sugar. On the contrary, it was stimulated from the very start. At the end of the first 48 hours on this agar *Ps. campestris* showed about twice as much growth, *Ps. phaseoli* "more growth," and *Ps. stewarti* four times as much growth as in the corresponding check tubes. On the seventh day *Ps. campestris* showed ten times as much growth as *Ps. hyacinthi*, and three times as much as in its own check tube (ten times as much on the sixteenth day). On this date *Ps. phaseoli* had made twice as much growth as in the check tube (ten times as much on the sixteenth day). On the same date *Ps. stewarti* had made at least five times as much growth as in the check tube.

In a second series of experiments with this agar *Ps. campestris* showed, on the third day, twice as much growth and *Ps. phaseoli* two and one-half times as much as there was in the check tubes. There was no retardation whatever.

(3) Addition of 17 per cent grape sugar retarded the growth of *Ps. campestris* and *Ps. phaseoli* (*Ps. stewarti* was not tried), but they overcame the injurious influence sooner than *Ps. hyacinthi*. If the volume of growth of *Ps. hyacinthi* on this agar on the sixth day be taken as 1, then that of *Ps. campestris* was 10 and that of *Ps. phaseoli* was 15 to 18.

(4) Addition of 23 per cent grape sugar entirely prevented the growth of *Ps. phaseoli* and seriously retarded that of *Ps. campestris*, but did not prevent it. On the contrary, when the retarding influence was overcome growth was greatly stimulated. On the seventh day this growth was only about one-fifteenth as much as in the check tube, or as on the 23 per cent cane-sugar agar. On the sixteenth day there was a marked increase of growth, but there was not one one-hundredth as much as in the corresponding tube of cane-sugar agar. On the thirtieth day the streak was 23 by 6 to 8 mm. On the thirty-seventh day growth had doubled, the streak now being 40 by 3 to 12 mm. The slime dissolved readily in water and consisted largely of chains 50 to 100 μ long. In a repetition of this series of experiments, 23 per cent grape sugar retarded but did not prevent the growth of *Ps. phaseoli*. The surface was rubbed with loops from agar cultures, but growth did not appear until the fourth day, and then only colony-wise.

(5) On the 9 per cent (acid) fructose agar *Ps. phaseoli* refused to grow. *Ps. campestris* obtained a precarious foothold, but grew only a little.

(6) Addition of 17 or 23 per cent cane sugar did not retard the growth of *Ps. campestris* or *Ps. phaseoli*, at least, not to any notice-

able extent. On the contrary, within a few days growth was enormously stimulated. If the volume of growth of *Ps. hyacinthi* on the 17 per cent cane-sugar agar at the end of eight days be reckoned as 1, then that of *Ps. campestris* was 2 or 3 and that of *Ps. phaseoli* was 3 or 4. On the 23 per cent cane-sugar agar, on the fourth day, the growth of *Ps. campestris* was five times as much as in the check tube, and that of *Ps. phaseoli* "vastly better." On the twelfth day the cultures of *Ps. campestris* and *Ps. phaseoli* resembled each other closely in color, general appearance, and amount of growth, which latter was ten times that in the corresponding tube of *Ps. hyacinthi*. On the thirty-sixth day the slime of *Ps. phaseoli* consisted of rods, doublets, fours, and many chains 50 to 120 μ long.

(7) The growth of *Ps. campestris* and *Ps. phaseoli* on the sugar agars was smooth, wet-shining, and often abundant enough and thin enough to flow like thick sirup on tilting the tubes. That of *Ps. hyacinthi* would never flow and was distinctly areolated, reticulated, wrinkled, or shagreened, as already described.

Sodium Acetate.

The stock (495) containing this substance was compounded as follows:

Distilled water, 400 c. c.
Dipotassium phosphate, 0.800 gram.
Magnesium sulphate, 0.040 gram.
Ammonium phosphate, 0.040 gram.
Sodium acetate, 2 grams.

This medium was filled into cotton-plugged test tubes and sterilized in the usual way. It was inoculated with *Ps. hyacinthi* very copiously from a young culture on coconut. It was under observation 5 weeks at 25° to 30° C., but growth progressed very slowly and was never anything more than feeble. At the end of the 5 weeks the fluid was still feebly clouded and there was no rim of germs or pellicle, but in the fluid on the wall of the tube were several hundred small, ragged, whitish flocks and on the bottom there was a pale yellow precipitate 5 mm. wide. The growth was not better than in Uschinsky's solution.

Ps. campestris also grew feebly in this fluid, and *Ps. stewarti* would not grow at all (only one test).

NUTRIENT STARCH JELLY WITH SUGARS, GUMS, AND ALCOHOLS.

Some comparative tests of these four yellow organisms as to color, rate of growth, etc., were made in tubes of slant nutrient, starch jelly to which 500 milligrams of special kinds of carbon foods were added—e. g., dextrin, lactose, maltose, etc. The growth in these tubes was compared with that in tubes of starch jelly to which the sugars, etc., were not added. My general conclusions are as follows:

TABLE V.—*Behavior of Ps. hyacinthi, etc., on nutrient starch jelly with various carbon foods.*

Substance added.	Organisms.			
	Ps. hyacinthi.	Ps. campestris.	Ps. phaseoli.	Ps. stewarti.
Lactose			A marked stimulating effect. Growth very copious, sirupy and with a magnificent production of the yellow pigment.	No increased growth.
Dextrin	A stimulating effect. Bright yellow. Growth several times as abundant as in the check tubes. Not sirupy.			No increased growth.
Maltose	Feeble at first, then several times as much as on check. Bright yellow.			No stimulating effect. Very feeble pale-yellow growth.
Galactose	A stimulating effect. Several times as much growth as in check. Slime very bright yellow.			Marked stimulating effect. As much growth or nearly as much as on cane sugar. 100 times as much growth as on the glycerine jelly.
Cane sugar	Copious bright-yellow growth. Several times as abundant as in check. After 128 days the starch immediately under the bacterial layer gave a marked reaction with iodine, blue and purple. Litmus reaction feebly acid.	Copious, smooth, wet-shining pale-yellow growth. Not much more abundant and not so yellow as finally in the glycerinated jelly.		Marked stimulating effect. A copious, smooth, wet-shining, buff-yellow, sirupy growth.
Mannit	No stimulating effect. Not more growth than in the check tubes.			No stimulating effect.
Glycerin	Growth feeble for some weeks as if retarded, color pale. After 128 days the whole surface of the slant (7 sq. cm.) was covered with a dense growth. The color was a uniform dull yellow, a little brighter than wax yellow. The entire surface was shagreened. The starch was not browned. It had lifted a little from the bottom on which was a small amount of yellow fluid due to the solvent action of the glycerol. Neither slime nor fluid were alkaline, both appeared to be neutral when wet, and the litmus was only slightly acid when dry. The slime was not sticky. The starch even immediately under the yellow layer reacted at once blue or purple with iodine.	Retardation of growth, of yellow pigment, and of diastasic action. After 24 days, however, a copious, sirupy smooth, wet-shining, rather bright yellow growth over whole slant, and culture then in marked contrast to *Ps. phaseoli* and *Ps. stewarti*.	Retards growth, diastasic action, and formation of yellow pigment (only one test). After 24 days not one one-hundredth as much growth as in the check or the lactose jelly and no distinct yellow pigmentation.	No stimulating effect, and apparently a distinct retardation.

TEMPERATURE EXPERIMENTS.

Thermal Death Point.

Some difficulty was experienced in determining accurately the thermal death point of *Ps. hyacinthi* owing to the slight variability in sensitiveness of individual rods. Considerable trouble was also experienced for some time owing to the frequent unaccountable failure of the germs to grow in some of the fluid cultures (see Sensitiveness to acids).

Most of these experiments were made in thin-walled test tubes 16 to 17 mm. in diameter, and containing exactly 10 c. c. of fluid (usually beef broth) entirely free from any trace of sediment or cloudiness. These tubes were inoculated in each case with big loops from fluid cultures only a few days old (1 to 11), and great care was taken in making the inoculations not to wet the walls of the tube above the fluid, and also to keep the tubes upright from first to last. The exposures were made by plunging the inoculated tubes into a hot-water bath nearly to their top, and keeping them in it at the given temperature for exactly 10 minutes. They were then removed, and either cooled quickly under running water or left to slowly acquire the temperature of the room. Duplicate tubes were always inoculated and maintained at the living-room temperatures for comparison. On two occasions poured plates were also made, using a large quantity of the culture fluid so as to determine more precisely the proportion of the germs killed by the heating.

The hot-water bath employed was the Ostwald-Pfeffer, using a very sensitive Roux metal-bar thermo-regulator, and a stream of compressed air for the motive power. The thermometer employed was a very sensitive one, belonging to a set made by Max Kaehler and Martini, of Berlin, and compared with the standard hydrogen thermometer of the International Bureau of Weights and Measures, Washington, D. C. With this apparatus, which keeps the water uniformly in motion, it was easy to maintain approximately constant temperatures for short periods.

The following is a detailed account of these experiments:

I. December 3: One tube of stock 204 inoculated with a large loop from tube 6 December 1. This tube was allowed to stand 1 hour and then plunged for 10 minutes into water at 54.30° C. Cooled at room temperature. Result: Under observation several weeks, but no growth.

II. December 3: One tube of stock 204 inoculated with a large loop from tube 6 December 1. This tube was allowed to remain 3 hours at room temperatures and then plunged for 10 minutes into water at 49.80° C. Cooled at room temperatures. Result: No growth. Tube under observation several weeks.

III. December 3, 1896: One tube of stock 204 (1:2 acid beef broth, i. e., no peptone or alkali added) inoculated with a large loop from tube 6 December 1, which

was a well-clouded,[1] 43-hour culture in stock 204. The germs were allowed to grow in the broth 1 hour, after which the tube was plunged for 10 minutes into water at 46° C. and then cooled at room temperatures. Result: Tube under observation several weeks, but no growth.

Check.—On December 3, at the same time as I, II, and III, another tube of stock 204 was inoculated with a large loop from tube 6, December 1, and left at room temperatures. Result: Growth was retarded, but not prevented. On the eighth day the medium was still clear, but on the thirteenth day the fluid was faintly clouded with a little precipitate and with good rolling clouds on shaking. This broth was not titrated, and consequently its grade of acidity was not known. It was feebly acid to litmus and contained a small amount of muscle sugar.

IV. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1 (a beef-broth culture inoculated with descendants of germs isolated from another hyacinth bulb). Each tube received a large loop of the fluid, and as the cultures were some days older, more germs than tubes I, II, III, and their check. After 1 hour both tubes were plunged for 10 minutes into water at 43.25° C., and then cooled at room temperatures. Result: December 11 both tubes are faintly clouded; December 17, moderately cloudy with rolling clouds on shaking and a small amount of yellow precipitate. The germs are not killed by 43° C., and are little, if any, retarded, the two check tubes clouding in about the same time and manner.

V. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1. In all respects a duplicate of IV, except that the water bath was 44.35° C. The tubes were cooled at room temperatures. Result: December 11 both tubes are faintly clouded; December 17, no pellicle, but a moderate amount of yellow precipitate and a good many small, roundish zooglœæ in the top layers of the fluid. These zooglœæ diffuse through the fluid on gentle shaking. Temperature of 44.35° C. does not kill or much retard growth. These tubes were compared with the 2 check tubes mentioned under IV.

VI. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1. In all respects like V, except temperature of water bath, which was 45.20° C. The tubes were cooled at room temperatures. Result: December 11, both tubes perfectly clear. On December 17, when next examined, the fluid in each tube was moderately cloudy, with distinct rolling clouds on shaking. Cloudiness easily visible without shaking. A little precipitate. Temperature of 45.20° C. does not kill, but considerably retards growth, the 2 check tubes (those mentioned under IV) being cloudy on December 11. The germs in tubes 6 and 7, December 1, were derived (as already stated) from different hyacinth bulbs.

VII. May 14: Six tubes of stock 217 (cauliflower broth feebly alkaline to neutral litmus), each inoculated with a loop from tube 5, May 10 (stock 218, a potato broth which came up slowly and was moderately cloudy, with rolling clouds on shaking). Four of these tubes were plunged for 10 minutes into water at 45.60° C., and two were held as checks. Result: The tubes were under observation for several weeks, but all of them, including the two checks, remained sterile. There was no apparent reason for the failure of the two checks, since the material used for inoculation was living (see VIII), and closely related organisms grew well in this broth, e. g., *Ps. phaseoli*.

VIII. On May 14, from two tubes of litmus neutral beef broth peptone agar, two poured plates were prepared in the following manner:

(1) One cubic centimeter of the well-clouded potato-broth culture (tube 5, May

[1] It is possible that part of this clouding may have been due to dead or feeble individuals derived from the original inoculation, which was probably from a solid culture.

10) was transferred by means of a sterile pipette to 10 c. c. of fluid agar (cooled to 41° C.), and after thorough shaking was poured into a sterile Petri dish.

(2) The remainder of the culture was then plunged for 10 minutes into water at 46.05° C.; it was then cooled at room temperatures for a few minutes and 1 c. c. taken out by means of another sterile pipette, put into another tube of melted agar (10 c. c. at 41° C.), and when thoroughly shaken poured into a second sterile Petri dish.

These two dishes were then kept at living-room temperatures and compared from time to time by turning them bottom up under the microscope. Result: May 18 (1) agar uniformly milky cloudy. Under the microscope innumerable small colonies are to be seen. Number of colonies estimated at 8,000 to 10,000 per field (Zeiss 16 mm. and 12 comp. oc.) (2) This plate was also milky cloudy, but the colonies were larger and not nearly so numerous, about 95 per cent having been destroyed by the heat.

These two plates were kept under observation for a week or two, but with no conflicting results.

IX. June 3, 1897, six tubes of stock 245, a beef broth made feebly alkaline to litmus by means of sodium carbonate, were selected for this experiment. Each was inoculated with a loop from tube 4, June 2, a 26-hour culture in stock 245, which was not yet distinctly clouded, but became so after a few hours. Four tubes were heated, but not until over an hour after inoculation (room temperature 28° C.) The temperature of the bath was unusually variable, ranging from 46.70° to 47.10° C., it being most of the time below 47° C.

Results: (1) Two of the tubes were cooled slowly at room temperature. These tubes were examined at intervals of a few days until July 29, but both remained clear.

(2) Two of the tubes were cooled quickly under running water. One of these tubes remained clear for 56 days, after which the experiment was discontinued. The other remained clear until the sixth day. It then became feebly clouded, and contained numerous small zooglœæ, most of the germs seeming inclined to pass at once into this state, i. e., growth was retarded but not all of the germs were killed. June 10, feebly clouded, zooglœæ larger, numerous, ragged. June 14, moderately clouded; slight rim on tube at level of liquid; the larger zooglœæ are distinctly yellow. June 16, well clouded with rolling clouds on shaking. Considerable distinctly yellow precipitate. A thin pellicle in shape of a delicate membrane thickly dotted with small zooglœæ is present. This membrane sinks on gentle shaking, breaking up into ribbons which are fine granular under ×6 Zeiss aplanat. June 28, copious yellow precipitate. The pseudo-pellicles have all settled. July 6, abundant yellow precipitate. Fluid nearly clear. On this date the other three (sterile) tubes were reinoculated from this tube, but they remained clear.

(3) Two of the tubes were kept as checks. One of them became contaminated with a white organism growing best on the bottom of the tube (Oospora?). The other remained clear until after the third day. On the fifth day it was distinctly but feebly clouded, and the surface layers contained small zooglœæ which streamed down cloudily on gentle shaking. June 9, clouded more than yesterday, but not heavily so. June 10, well clouded with considerable yellow precipitate. June 14, a pellicle consisting of yellowish more or less united zooglœæ. June 16, well clouded with rolling clouds on shaking. No new pellicle. The broken one (shaken down on the 14th) has not gone to pieces, but lies on the bottom with hundreds of tiny zooglœæ embedded in it very regularly. June 28, a copious yellow precipitate. July 6, fluid nearly clear, i. e., becoming exhausted of nourishment; otherwise as before. July 29, washed out; precipitate yellow.

X. On June 3 three tubes of stock 244b (+20 gelatin) were converted into 3 poured plates as follows:

(1) One cubic centimeter of the cloudy fluid from tube 4, June 2 (see IX), was

transferred to 10 c. c. of gelatin at 30° C., shaken, and poured into a sterile Petri dish to form the check plate.

(2) The remainder of the culture (approximately 9 c. c.) was then plunged for 10 minutes into water at 46.50° to 46.60° C., and another 1 c. c. immediately pipetted out into another tube of 10 c. c. of gelatin at 30° C., shaken, and poured into a second Petri dish.

(3) The culture was then allowed to cool to room temperatures after which 1 c. c. was pipetted into another 10 c. c. of gelatin at 30° C., shaken and poured into a third Petri dish.

These 3 dishes were then put into the cool box, where they were kept at 12° to 16° C., and examined and compared from time to time in the same way as the dishes of agar.

Results: (1) Colonies to the number of 2,000 to 3,000 per field (Zeiss 16 mm. and 12 comp. ocular) appeared in this dish. (2) More than 80 per cent of the germs were destroyed by the heat, i. e., there were only 200 to 600 colonies per field in this dish. (3) About 400 colonies per field appeared in this plate.

The culture from which these 3 plates were inoculated was made with a single loop from a broth culture 11 days old and this fact, together with its own age (28 hours at 24° to 28° C.), precludes the idea that spores played any part in the results obtained.

XI. February 3, 1898: Six tubes of alkaline beef broth, stock 286b (stock 286 consisted of the broth from 1,000 grams of minced lean beef covered with 1,500 c. c. distilled water and left in the ice box 24 hours. The fluid was finally made up to 2,000 c. c., titrated, and divided into four equal parts. Stock 286b received enough $\frac{2N}{1}$ NaOH to render it exactly neutral to phenolphthalein, i. e., strongly alkaline to neutral litmus paper). These tubes were of Weber's resistant glass, 169 by 17 mm., and very thin walled. Each received exactly 10 c. c. of the broth, which, from previous tests, the germ was known to grow readily, even when added in very small quantities. Each of these 6 tubes was inoculated with a drop of fluid from tube 1, Jan. 29, a beef broth which was nicely clouded with good rolling clouds on shaking. This broth had been clouded about 55 hours, but showed as yet very little precipitate and no pellicle or zoogloeæ. As much fluid was put into each tube as could be lifted out on a medium sized (2 mm.) loop and 5 or 6 cm. of wire above it, i. e., an enormous number of germs, as microscopic examination showed. About 15 or 20 minutes after inoculation 4 of the tubes were plunged into the hot water, while the other 2 were held as checks. The exposed tubes were put well down into the bath so that the surface of the broth was 5 to 8 cm. below the surface of the water, which was in constant motion. The exposure was exactly 10 minutes. On removal, 2 of the tubes were cooled immediately under flowing water, while the other 2 were allowed to cool gradually at room temperature (23° C.). All were then screened from the diffused light of the room and set away at room temperatures which varied from 15° to 25° C. The temperature of the water bath at the beginning was 47.80° C., falling slowly to 47.58° C. at the end. During the middle 8 minutes the range of temperature was from 47.70° C. to 47.60° C. These tubes were under observation 33 days.

Results: (1) Checks. Both tubes clouded inside of 48 hours and passed through a normal course of growth. (2) Cooled quickly. Both tubes remained perfectly clear. (3) Cooled slowly. Both tubes remained perfectly clear.

XII. February 3, 1898: This experiment was in all respects a duplicate of the preceding, except that the water was a trifle cooler and that after inoculation the tubes were allowed to stand one-half hour before plunging. The temperature of the water was 47.45° C. at the beginning and 47.17° C. at the close. After one minute the temperature of the bath fell to 47.30° C. and during the next 5½ minutes it gradually fell to 47.20°. During the remaining 3½ minutes the temperature fluctuated between 47.16° C. and 47.18° C., being at the latter point most of the time.

Results: (1) Checks. Both tubes clouded inside of 48 hours and developed normally. (2) Cooled quickly. Both tubes remained perfectly clear till the end of the experiment (33 days). (3) Cooled slowly. Both tubes remained clear until the ninth day. Then one of them became very feebly clouded and gradually passed through the same changes as the check tubes, but never caught up with the latter. The other tube continued clear till the end of the experiment.

As a result of these experiments we may conclude that exposure of *Ps. hyacinthi* for 10 minutes to a temperature of 43° C. does not appreciably retard growth; 44° retards growth slightly; 45° retards considerably; 46° to 46.50° destroys the greater part of the organisms; 47.17° to 47.45° (mostly 47.20° to 47.30°) destroys almost all; 47.58° to 47.80° (mostly 47.60° to 47.70°) destroys all.

The thermal death point, therefore, under the exact conditions named, may be recorded as approximately 47.50°, but a majority of the rods are killed at 46.50° C.

Probably some of the rods are destroyed by 10 minutes' exposure to temperatures as low as 45° or 45.50°. Exposures for much longer periods to temperatures a few degrees lower, e. g., 7 days at 40° C., have the same effect, as may be seen from what follows.

The thermal death point of *Ps. stewarti* in +15 beef bouillon is approximately 53° C. In Uschinsky's solution it is a little higher.

The thermal death point of *Ps. phaseoli* is approximately 49.50°, and that of *Ps. campestris*, is 51.50°.

Maximum Temperature for Growth.

The maximum temperature at which *Ps. hyacinthi* will grow in favorable media is 34° to 35° C., the exact temperature limit varying somewhat with the medium used and with the heat resistant power of individual rods. This conclusion rests on the following experiments, which were made in a Rohrbeck thermostat, covered with thick haircloth and provided with a large water reservoir, so that the culture chamber is not quickly sensitive to changes in gas pressure or in the temperature of the room.

(1) In stock 244c (0 gelatin), kept in the thermostat at 40° C., there was no growth whatever, and none appeared when this tube was removed from the thermostat at the end of 7 days and kept at room temperatures for an additional 38 days. This tube was inoculated with a very large loop from a beef-broth culture, which had been cloudy for 6 days. In a second tube of this gelatin, inoculated from the same culture at the same time and in the same manner, but kept throughout at room temperatures of 24° to 34° C. (mostly 25° to 29°), the organism developed normally, clouding the fluid in 24 hours.

(2) Three potato cylinders (stock 246) were inoculated at the same time and from the same culture as the 2 tubes of gelatin. One of these was put into the thermostat at 40° C. and the other 2 were kept at room temperatures.

Result: In each of the 2 check tubes the organism developed normally, the first distinct sign of yellow growth being visible in about 47 hours. No trace of growth appeared in the tube which was put into the thermostat, although a very large loop of

broth was used in making the inoculation, and there was plenty of water in the bottom of the tube. After 7 days this tube was removed from the thermostat and kept in the dark at room temperatures for 38 days, but no growth ensued.

(3) Three well-plugged tubes of 1:2 moderately litmus alkaline beef broth (stock 245) were inoculated at the same time from the same tube, and in the same way. Two of these were put into the thermostat at 40° C. and the other was kept at room temperatures.

Result: The check tube clouded on the fourth day and passed through a normal course of development. The tubes in the thermostat remained perfectly clear until the end of the experiment (45 days).

(4) Three tubes of cauliflower broth (stock 217), which by long standing had dried out one-fifth (2 c.c.), were also inoculated at the same time from the same culture and in the same way. Two of these tubes were kept at room temperatures and the third was put into the thermostat at 40° C.

Result: One of the check tubes clouded on the third day, the other some time between the fourth and seventh day. Both developed a yellow pellicle and threw down a yellow precipitate. The tube in the thermostat was under observation 45 days, but there was no growth.

(5) Three tubes of 1:2 acid beef broth (stock 204) were each inoculated with a large loop from a beef-broth culture of *Ps. hyacinthi* 7 days old. This culture, which was moderately cloudy, showed many small zoogloœ floating in the fluid, and on the bottom a small amount of decidedly yellow precipitate. Two of these tubes were put into the thermostat at 36° to 38° C. and the third was kept at room temperatures (mostly 21°).

Result: On the third day the check tube became feebly clouded and contained many tiny zoogloœ. On the eleventh day this tube was moderately cloudy, showed a yellow precipitate, and bore on the wall of the tube at the surface of the fluid a yellow rim of loosely adhering zoogloœ. An agar culture inoculated from the same tube at the same time and kept at room temperatures also developed normally. The tubes in the thermostat remained free from bacterial growth as long as the experiment continued (22 days).

(6) Three tubes of resistant glass, each containing 10 c. c. of strongly alkaline beef broth (stock 286b, neutral to phenolphthalein), in which *Ps. hyacinthi* was known to grow well, were each inoculated with a loop from a clouded tube of alkaline beef broth 6 days old. After remaining for an hour at room temperatures, 2 of these tubes were put into the thermostat and kept at 35° to 36.35° C. during the first 5 days, then at 32° to 33.50° for 24 hours, and afterwards at 34.15° to 35.35°. The third tube was kept in the dark at room temperatures ranging from 18° to 23° C., except on one day when the room temperature fell to 8° C. Each of the tubes put into the thermostat received a large loop of the cloudy broth; the tube left at room temperatures received a smaller loop of this broth, i. e., not one-fourth as many germs.

Result: In 43 hours the check tube was distinctly clouded. On the fourth day it was well clouded, free from zoogloœ, and showed some yellow precipitate. The other 2 tubes remained clear as long as they were left in the thermostat. One was removed on the thirteenth day and left for 24 days at room temperatures (mostly 22° C.), but no growth ensued. The other was removed on the sixth day and left at room temperatures 31 days, but no growth ensued. At the close of the experiment the tubes still contained 8 c. c. of broth, i. e., the concentration was not beyond what this organism bears readily.

(7) Three cylinders of sugar beet (stock 292) were inoculated at the same time and from the same tube as the preceding, each tube receiving a large loop of the cloudy fluid. Two of these tubes were put into the thermostat along with the beef broth (6), and the third was kept at room temperatures.

Result: The check tube showed no growth at the end of the fourth day, i. e., there

was some retardation. On the sixth day, when next examined, there was a distinct yellow growth over a large part of the cylinder. On the eighth day this growth was bright yellow and copious. The development of this culture was normal, and continued for a month or more. The 2 tubes put into the thermostat remained free from bacterial growth. Both were taken out on the thirteenth day and left at room temperatures (19° to 26° C.) for 54 days, but there was never any growth.

(8) Four well-plugged tubes of resistant glass, containing 10 c. c. of strongly alkaline beef broth (stock 286b), which had evaporated to 8 c. c. by long standing, were each inoculated with a large loop from a beef-broth culture of *Ps. hyacinthi* 48 hours old, which had been inoculated copiously from a solid culture and was cloudy from growth. Two of these tubes were kept in the dark at room temperatures of 20° to 25° C. The other 2 were put into the thermostat at 33.35° to 35.58° C. (mostly 34.32° to 34.55°) during the first 8 days, and after that at 32.45° to 35.55°.

Result: The 2 check tubes clouded in 48 hours and developed normally. The other 2 tubes remained clear as long as they were kept in the thermostat—37 days for one and 13 days for the other. The latter was removed on the thirteenth day and kept at room temperatures for 24 days, but no growth ensued. The germs were dead, however, in each tube considerably in advance of the thirteenth day, for 2 tubes of the same beef broth which were inoculated therefrom on the eighth day, using large loops, and left in the dark at room temperatures, remained entirely free from growth as long as the experiment continued (29 days).

(9) Two cylinders cut from a yellow turnip and steamed in the usual amount of water were inoculated at the same time, from the same culture, and in the same copious manner as the preceding. One of these was put into the thermostat and the other was kept at room temperatures.

Result: The check tube showed a distinct yellow growth on the third day. On the fifth day this growth was copious and typical for *Ps. hyacinthi*. The tube in the thermostat showed no growth on the fifth day and was then reinoculated with a large loop of yellow slime from the check tube. The tube was then shaken until the slime was washed over the cylinder and dissolved in the fluid, and the yellow color invisible. The tube was then put back into the thermostat. In 26 hours there was a slight yellow growth on the upper part of the cylinder (temperature 34.45°, falling slowly to 33.35° C.). Two days later there was, apparently, no increase of growth (temperature 34.53° a. m., 34.15° p. m., 34.32° a. m.), and not one one-hundredth part as much growth as in a tube inoculated at the same time for comparison. On the eighth day (temperatures 34.40° to 35.55° C.) growth was very scanty and the color scarcely visible. The amount of growth at this time was not one three-hundredth as much as in the check tube held at room temperatures. On the twelfth day after this reinoculation growth had increased a little, but was still very feeble and certainly not one one-hundred and fiftieth as much as the same culture would have given at room temperatures. During these last 4 days the thermostat was considerably cooler, the temperature of the culture chamber ranging from 32.45° to 34.45° C., and being most of the time below 34°. After 49 days in the thermostat a tube of alkaline beef broth was inoculated very copiously from this tube and left at room temperatures 27 days, but no growth ensued, i. e., the vegetative rods were dead and no spores were present.

(10) Two cylinders of steamed sugar beet were inoculated at the same time, from the same culture, and in the same manner as in the two preceding experiments. One of these tubes was put into the thermostat and the other was held at room temperatures.

Result: On the fifth day there was no visible growth in either tube and both were reinoculated very copiously with the solid slime from a turnip culture 5 days old (the check of series 9). The tube which came from the thermostat was shaken thoroughly before replacing, so that if there were any subsequent growth it might not be

confused with any undissolved slime used in making the inoculation. At the end of 26 hours there was a slight growth on the cylinder in each tube. On the third day, in the thermostat (temperatures 34.45° p. m., 34.35° a. m., 33.35° p. m., 34.53° a. m., 34.15° p. m., 34.32° a. m.) the germs covered 2 sq. cm. on one side of the cylinder. This growth was plainly yellow but extremely thin. On the fifth day (temperatures 34.32° p. m., 35° a. m., 34.40° p. m., 34.85° a. m.) there seemed to be a slight increase in growth. This growth was very thin, distinctly yellow, not smooth, and rather dry, i. e., not wet-shining. In the check tube there was from 10 to 20 times as much growth, but not as much growth as there should have been, owing to the fact that the check cylinder was rather dry. On the eighth day (temperatures 34.85° a. m., 34.55° p. m., 35.55° a. m., 35.45° p. m., 34.83° a. m., 34.65° p. m., 34.95° a. m.) there was some increase, the growth being distinctly yellow, but too thin to hide minute irregularities of the substratum. The volume of growth at this time was not one-fiftieth that in the check tube. Examined microscopically, this growth consisted of zooglœæ, short rods and long rods. The short rods were single, in doubles, or in fours; the long rods were slender threads, 10 to 20 or more times the length of an ordinary rod. These threads were numerous and their segments were not well defined. No involution forms were observed or any bodies suggestive of spores. On the twelfth day (temperatures 34.05° a. m., 33.35° p. m., 34.45° a. m., 33.35° p. m., 32.75° a. m., 32.45° p. m., —— a. m.) the growth was meager, thin, dull yellow, and its surface was shagreened. There was no yellow slime in the water, but the germs on the cylinder out of the water appeared as if still growing, although very slowly. After 49 days in the thermostat a large loop of slime from this tube was removed and put into alkaline beef broth. This tube was kept at room temperatures for 27 days, but no growth ensued.

(11) This experiment was undertaken to see if cultures started at room temperatures would not do better when put into the thermostat than those which had been inserted soon after inoculation. For this purpose I selected a tube of alkaline beef broth, which had been kept as a check on series No. 8, and a tube of yellow turnip, kept as a check on series No. 9. The turnip culture was put into the thermostat on the fifth day, at which time there was a copious, yellow, wet-shining, homogeneous-looking growth covering most of that part of the cylinder out of the water. The tube of beef broth was put in on the eighth day, at which time the fluid was moderately cloudy and had thrown down a little yellow precipitate, but had not yet developed any pellicle, rim of germs, or zooglœæ. The temperatures were 34.15° to 35.55° during the first 9 days (once as low as 33.35°) and then 32.45° to 34.45° C. There was no exact check tube for the turnip, but a transfer was made from it into another tube of the same medium; for comparison with the beef broth the other check tube of series No. 8 was used.

Result: (a) The beef broth in the thermostat at once fell behind the check tube in growth. On the fifth day the clouding appeared to be feebler than on the start and the trifling precipitate had increased proportionately to the decrease in clouding, but scarcely more. The check tube was distinctly cloudier. On the ninth day there was no increase of precipitate. On the twenty-ninth day there was no pellicle, no rim of germs, no zooglœæ, and not more precipitate than on the fifth day, i. e., there appeared to have been no growth whatever during the whole time of the exposure. On this date the check tube was uniformly clouded, showed a yellow rim, and had thrown down a yellow precipitate 12 mm. broad and 2 mm. deep. On the twenty-ninth day a large loop of fluid was taken from the tube in the thermostat and put into a sterile tube of the same beef broth. This tube was under observation 17 days, in conditions very well suited for growth, but no growth ensued. At the end of 46 days in the thermostat this experiment was repeated, inoculating copiously into alkaline beef broth diluted with distilled water. The tube was kept at room temperatures and watched for 27 days, but no growth ensued, i. e., no spores were pres-

ent. (b) On the fifth day in the thermostat the slime on the turnip cylinder was still wet-shining, but it was not as homogeneous looking, being uniformly mottled lighter and darker yellow. On the eighth day the culture was less vigorous and the substratum had browned slightly. The slime was now examined microscopically for several hours. It consisted of the ordinary short rods and of slender threads which were of the same diameter as the rods but were often 50 times as long. These threads were numerous. No involution forms were observed nor any bodies resembling spores. On the twelfth day the culture was in a much worse condition. Growth had ceased and the slime out of the water had so much dried out that the substratum under it was now visible. Nineteen days after this date the turnip cylinder which had been inoculated from this tube and kept at room temperatures was still covered with a thick, smooth, wet-shining, homogeneous-looking, pale yellow layer of slime, entirely hiding the substratum. The fluid in the bottom of the tube was also grown full of the slime, which was not the case with the culture in the thermostat. After 49 days in the thermostat a large loop from this tube was put into alkaline beef broth and watched at room temperatures for 27 days, but no growth ensued.

(12) Two steamed cylinders of carrot (stock 290), standing in several cubic centimeters of distilled water in tubes of resistant glass, were each inoculated with a large loop of the yellow slime of *Ps. hyacinthi* from recent growths in a turnip culture 5 days old. These tubes were then shaken until the slime was dissolved in the water and washed over the cylinder. One of the tubes was put into the thermostat at 33.35° to 35.45° C. (mostly 34.35° to 35°) and the other was held at room temperatures.

Result: On the third day the check tube showed a plentiful yellow growth, covering nearly all of one side of the long cylinder. On the fifth day this growth was dense enough to hide the orange color of the substratum. The tube in the thermostat, on the eighth day, showed no growth whatever, although it still held 2 c. c. of water and was consequently moist. This tube was now removed to room temperatures of 19° to 25° C. On the fourth day thereafter a copious, smooth, wet-shining, homogeneous-looking, bright yellow growth, dense enough to hide the substratum, covered about 3 sq. cm. of the inoculated cylinder.

(13) Two cylinders from a yellow, flat-bottomed turnip, prepared in the same way as the carrot, were inoculated at the same time as the latter and from the same culture, each tube receiving a large loop of the yellow slime. These tubes were then shaken until the slime was dissolved in the water and spread over the cylinder. One of the tubes was held at room temperatures and the other was put into the thermostat. The tube in the thermostat contained several cubic centimeters of water; the check tube contained only a small amount of water.

Result: On the third day, in the check tube, there was a copious, smooth, wet-shining, yellow growth over nearly the entire cylinder. On the fifth day this growth had become more abundant, covering the whole cylinder and filling up the small amount of fluid in the bottom of the tube. The other tube was left in the thermostat 8 days at 33.35° to 35.55° (mostly 34.35° to 35°), during which time no growth was visible either to the naked eye or with a Zeiss × 6 aplanat. The tube was now removed to room temperatures of 20° to 25° C. On the fourth day after this removal two-thirds of the cylinder (all out of the water) was covered with a copious, yellow, smooth, wet-shining, homogeneous-looking bacterial layer, which developed normally for *Ps. hyacinthi*.

(14) Four tubes of 1:2 acid beef broth (stock 286a, acidity + 25), originally holding exactly 10 c. c., but dried out about one-fifth by long standing and consequently more acid than the original stock, were each inoculated with two large loops from an alkaline beef broth culture of *Ps. hyacinthi* 10 days old. This culture was uniformly clouded, and showed considerable yellow precipitate, but there were no zoogloœ and the rim of germs was only commencing to form, i. e., the fluid was crowded full of living germs and in excellent condition for use. Two of the tubes

inoculated therefrom were set away in the dark at room temperatures of 19° to 25° C. (mostly 21° to 23°). The other two were put into the thermostat at 34.55° to 35.55° for the first 4 days and then at 32.45° to 34.45° C.

Result: The check tubes were feebly clouded on the third day. They were first examined at the end of 72 hours, and probably clouding could not have been detected more than 6 or 8 hours earlier. These two cultures passed through a normal development. The other tubes were left in the thermostat 27 days, during all of which time they remained perfectly clear. On the twenty-seventh day both were removed to room temperatures and watched for 6 weeks, but they never clouded. When removed from the thermostat each tube still contained about 6.5 c. c. of fluid.

The following inferences respecting *Ps. hyacinthi* appear to be warranted by these experiments:

(a) The organism will not grow on any medium at 40° C., and after 7 days exposure to this temperature it will not grow at any temperature. Probably a much shorter exposure to 40° C. would kill it.

(b) The organism will not grow in unneutralized (acid) beef broth at 36° to 38° C., and consequently it is not likely that it will prove pathogenic to warm-blooded animals.

(c) The organism will not grow in strongly alkaline beef broth at 35° to 36.35° C., and after 6 days' exposure to this temperature it will not grow at any temperature.

(d) The organism will not grow on sugar-beet cylinders at 35° to 36.35° C., and after 13 days' exposure to this temperature will not develop at any temperature.

(e) The organism will not grow in strongly alkaline beef broth at 34.15° to 35.58° C., and after 8 days' exposure to this temperature it will not grow at any temperature.

(f) When inoculated very copiously from a young solid culture, the organism grew scantily on yellow turnip at 33.35° to 34.45° C.

(g) When inoculated very copiously from a young solid culture, the organism grew very feebly on sugar beet at 34.15° to 35° C.

(h) Growth already well under way in strongly alkaline beef broth and on yellow turnip was stopped at 34.15° to 35.55° C.

(i) In 8 days the organism made no visible growth on steamed carrot at 33.35° to 35.45° C., but all of the germs were not killed.

(k) In 8 days the organism made no visible growth on yellow turnip at 33.35° to 35.55° C., but all of the germs were not killed.

(l) In 27 days the organism made no growth in unneutralized (acid) beef broth at 34.55° to 35.55° C., and all were dead before the twenty-seventh day.

Ps. stewarti refused to grow at 40° C., in Uschinsky's solution and in strongly alkaline beef broth (0 of Fuller's scale). It grows in the thermostat at 36° to 37° C., on most media, but not so well as at room temperatures of 24° to 25° C. *Ps. campestris* did not grow at 40° C., and grew not at all or very feebly at 37° to 38° C.—i. e., about as *Ps. hyacinthi* grows at 34° to 35° C.

Optimum Temperature for Growth.

No special experiments have been instituted to determine at what temperature growth of *Ps. hyacinthi* is most rapid, but from a careful collation of the records of several hundred cultures made during the past four years and kept at room temperatures—i. e., of all cultures which were examined frequently enough during the first few days of growth, and for which the necessary temperature records were set down—I find that, on good media, growth was slow at 10° to 12° C., moderate at 18° to 25° C., and fast (for this organism) at 28° to 30° C. These cultures were instituted at all seasons of the year, and sometimes for several days together the room temperature would be nearly stationary—e. g., at 18°, 25°, or 30° C. In a few instances I have thus been able to compare at different temperatures the rate of growth when the inoculations were made with the same amount of material taken from cultures of the same age and kind

Using these records, therefore, as a basis for judgment, the optimum temperature for growth may be placed at 28° to 30° C.

Minimum Temperature for Growth.

On very favorable media this is believed to be about 4° C. for *Ps. hyacinthi*. Only four sets of experiments have been made. (1) On a sugar beet cylinder inoculated copiously with bright yellow slime from a starch jelly culture 8 days old and kept in the ice chest at 10° to 12° C. (temperature possibly at times as low as 7° or 8° C., but never lower) no visible growth appeared in 12 days. The tube was now removed to room temperatures. Five days afterwards there was a distinct yellow growth covering more than 2 square centimeters of the surface.

(2) A tube of unneutralized 1:2 beef broth (stock 204), inoculated with a large loop from a well-clouded beef broth culture 7 days old and put into the ice chest at 10° to 12° C., was clouded very feebly at the close of the fifth day. A check tube at 21° C. clouded feebly in 67 hours.

(3) Two freshly prepared cylinders of coconut, standing in test tubes in an abundance of distilled water, were each inoculated with approximately 1 c. mm. of yellow slime from a coconut culture 4 days old. These tubes were put into the ice chest. In 42 hours there was a slight but distinct growth in each tube, the temperature, however, had been higher than was anticipated—i. e., 10° to 15° C. These tubes were now shaken for 10 minutes—i. e., until all trace of the yellow growth was washed off and dissolved in the fluid. They were then put back into the chest with a larger quantity of ice. On June 2, 4 p. m. (after 54 hours), there was a slight growth in each tube, although the temperature had remained under 8° C. On June 3, 9 a. m. (tem-

perature 8.2° C.), there had been some further growth. On June 4, 9 a. m. (temperature 8.5° C.), there was a distinct increase of growth over what was present 24 hours earlier. One of the two tubes was now removed to room temperatures of 25° to 26° C. During the next 26 hours the growth in this tube doubled. During the same period there was a slight growth in the other tube (temperature 8.5° C.). At this time, in this tube, a bright yellow growth covered more than 1 sq. cm. of the surface where 5 days before (after the shaking) no growth whatever was visible. All of this growth took place between 7.4° and 9° C., the temperature most of the time during the 5 days ranging between 7.5° and 8.5° C.

(4) Four tubes of strongly alkaline beef broth (stock 382 neutral to phenolphthalein) were each inoculated with a 3 mm. loop from a well-clouded beef broth culture 3 days old. One of these tubes was held at room temperatures of 20° to 25° C. This culture was moderately clouded on the third day and passed through a normal growth. The other 3 tubes were placed in the ice chest for 18 days at 2.8° to 4.5° C. (mostly 3° to 4° C.), during the whole of which time they remained perfectly clear. On then removing them to room temperatures they clouded in 16 hours at 21°–23° C. The rapidity with which they clouded when removed from the ice box suggests that the bacteria grew slightly at times while exposed to the low temperature.

The minimum temperature of *Ps. campestris* is not known. It lies below 7° C. The minimum temperature of *Ps. stewarti* is not known exactly, but it is believed to be a degree or two higher than that of *Ps. hyacinthi* for the following reason: Tubes of *Ps. stewarti* were exposed in the ice box at 2.8° to 4.5° C., along with those of the hyacinth germ. There was no clouding in 18 days, and on removing to room temperatures the tubes were not clouded until the third or fourth day, and then only feebly. The check tubes clouded on the second and third days. The fluids used were Uschinsky's solution and an alkaline beef broth (stock 382). Each tube was inoculated with one 3-millimeter loop from a young fluid culture (3 days old). The date of clouding on removal indicates clearly that, contrary to the case of *Ps. hyacinthi*, there had been no growth whatever during the 18 days' sojourn of the tubes in the ice chest.

FORMATION OF ACIDS.

With exception of the production of a small amount of acid from ethyl alcohol (probably acetic acid), the formation of acids by *Ps. hyacinthi* is rather obscure, in spite of all the attention I have given to it. At times, especially when small quantities of the carbohydrate were used, no acid was detected from the growth of this organism in the presence of sugars. Even when large quantities of the various sugars were used there was no prompt change from alkaline or neutral

to acid. After some weeks, however, many of these cultures changed from alkaline to neutral, and others became decidedly acid, and the acidity increased on concentration by boiling rather than diminished. It would seem, therefore, that a small quantity of some non-volatile acid is formed by this organism from a variety of substrata, but that the formation of this acid is in no way associated with facultative anaerobism or with the production of gas.

The other yellow organisms, so far as tested, behaved in the same way as *Ps. hyacinthi*, so far as relates to the slow development of a non-volatile acid in the presence of certain sugars and of certain vegetable substances rich in sugars.

FORMATION OF ALKALIES.

Feebly acid or neutral culture media of various kinds were finally rendered alkaline by *Ps. hyacinthi*, but not rapidly so, and all the tests instituted lead me to the conclusion that this organism is a relatively feeble alkali producer. This alkali is volatile, and a part of it, at least, is undoubtedly ammonia. Neutral or acid reactions were observed in the following old and very old cultures: Carrot, sugar beet, sweet potato, yellow globe turnip, grape sugar agar, cane sugar agar, nutrient starch jelly with cane sugar, nutrient starch jelly with glycerin. The following culture media became and remained alkaline: Potato, cocoanut, ordinary nutrient agar, salted peptone water, milk, milk with grape sugar, milk with methyl alcohol, milk with glycerin, hyacinth broth.

The results obtained by special tests are given under the following heads:

Rosolic Acid Test.

The action of *Ps. hyacinthi* on rosolic acid was tested in Dunham's solution. To each 100 c. c. of this salted peptone water was added 1 c. c. of a solution made of 0.5 gram rosolic acid; 20 c. c. distilled water; 80 c. c. absolute alcohol. The alkali in the peptone (Witte's) made this culture medium too red, and the fault was remedied by adding to each 90 c. c. of the solution 6 drops of $\frac{2}{1}^{N}$ HCl, which rendered the medium yellowish and suitable for the experiment. The results obtained with this organism and with others used for comparison are given in the following table:

TABLE VI.—*Behavior of Ps. hyacinthi and other bacteria in salted peptone water with rosolic acid and HCl (stock 493). Original color of fluid pale orange yellow.*

Organism.	Growth.	Change in color.			
		First week.	Second week.	Third week.	Fourth week and later.
Ps. hyacinthi	Moderate after a time, but none visible on 3d day, and feeble on 6th.	3d day, no change in color; 6th, no reddening.	9th day, paler than check; 11th, color gone.	16th day, all the yellow color has gone and no red has come; fluid colorless but clouded; 21st, colorless.	28th day, fluid colorless or faint rose color when looked through endwise; precipitate rosy or salmon; 37th, no red color—colorless in comparison with check; 56th, no red color.
Ps. campestris	Good; feebly clouded on 3d day; not heavily clouded on 9th.	3d day, no change in color; 6th, no reddening.	9th day, a distinct change to pink; this was first observed on 8th.	16th day, fluid geranium red, the only culture showing any distinct red; 21st, as on the 16th.	28th day, fluid deep red, approximately Ridgway's scarlet; 37th, as on 28th, but color deeper, i. e., poppy red; 56th, the deep red color persists.
Ps. stewarti	Good; well clouded on 3d day.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; 11th, no reddening.	16th day, color not deeper than check, but rosaceous rather than yellowish; 21st, slight, if any, change since 16th.	28th day, a decided change; the fluid is now plainly pale red, i. e., R.'s flesh color, with a trifle of pink in it; color about one-fourth as deep as in tube of Ps. campestris; 37th, much more color; it is now geranium red. This medium alone would serve to separate Ps. stewarti from Ps. hyacinthi, if the results here obtained prove constant; 56th, the color now lies between scarlet vermilion and geranium red.
B. pyoc. pericarditidis	Good; well clouded on 3d and 9th days.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; like check in color; 11th, no reddening.	16th day, like Ps. stewarti, colonies on the rim are yellowish-salmon; 21st, no distinct change.	28th day, red color developing, about one-half as deep as tube of Ps. stewarti, i. e., R.'s pale flesh color; 37th, the color has deepened; the fluid is now deep flesh color, i. e., a deeper color than R.'s No. 18; 56th, color pale geranium red, i. e., much deeper than it was.
B. coli (a strong indol producer from Dr. Theobald Smith).	Good; well clouded on 3d and 9th days.	3d day, no change in color; 6th, no reddening.	9th day, resembles check in color; 11th, no reddening.	16th day, like Ps. stewarti and B. pyoc. pericard.; 21st, color not deeper than check, but slightly rosaceous, in marked contrast to Ps. campestris.	28th day, fluid a pale red, same depth and same shade as in tube of B. pyoc. pericard.; 37th, fluid now pale flesh color; 56th, color slowly deepening; it is now midway between flesh color and geranium pink.

TABLE VI.—*Behavior of Ps. hyacinthi and other bacteria in salted peptone water with rosolic acid and HCl (stock 493). Original color of fluid pale orange yellow.*—Continued.

Organism.	Growth.	Change in color.			
		First week.	Second week.	Third week.	Fourth week and later.
B. amylovorus	Good; well clouded on 3d and 9th days; moderately cloudy on 16th.	3d day, no change in color; 6th, no reddening.	9th day, resembles check in color; 11th, no reddening.	16th day, color same as in tube of Ps. stewarti; 21st, color not deeper than in check tube, but feebly rosaceous instead of yellow.	28th day, as on 21st; 37th, distinctly paler than B. coli, same depth of color as check, and nearly the same orange yellow, but with a trifle of rose in it; 56th, a decided change in color; the fluid is now midway between flesh color and geranium pink.
B. carotovorus	Good; well clouded on 3d, 9th, 16th, and 28th days.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; if any change, paler than check.	16th day, color gone; fluid as colorless as in tube of Ps. hyacinthi; 21st, as on 16th.	28th day, no color except in the precipitate, which is salmon. This organism and Ps. hyacinthi are alike in having destroyed the original yellowish color of the fluid without the production of any red color, and in having a stained precipitate; 37th, fluid colorless, precipitate orange brown; 56th, fluid colorless.

ACID FUCHSIN TEST.

The action of *Ps. hyacinthi* on acid fuchsin was tested in peptone water. The culture medium was prepared as follows:

200 c. c. distilled water.
2 gm. Witte's peptonum siccum.
4 c. c. acid fuchsin water.
10 drops $\frac{2N}{1}$ HCl (to counteract the alkalinity of the peptone).

The acid fuchsin water consisted of 150 mg. of Grübler's Fuchsin S. (after Weigert) dissolved in 30 c. c. of distilled water.

The tubes each contained 10 c. c. of the rose-red fluid. They were inoculated on March 21. Tubes 1, 2, and 3 were inoculated from fluid cultures; tubes 1′, 2′, and 3′ were inoculated from solid cultures. The results obtained with *Ps. hyacinthi* (tubes 1 and 1′), *Ps. campestris* (tubes 2 and 2′), *Ps. stewarti* (tubes 3 and 3′), *B. pyoc. pericarditidis* (tube 4), *B. coli* (tube 5), *B. amylovorus* (tube 6), and *B. carotovorus* (tubes 7 to 10) are summarized below:

March 24.—Slight variations in color, but each tube paler than the check tubes.

March 27.—Nos. 1′, 2, 2′, 3′, 4, and 5 are much alike in color. They have faded considerably; i. e., they are now rose color. Nos. 1, 3, and 6 are deeper red. None are colorless, but all except 7–10 are paler than on the twenty-fourth.

March 30.—There has been a marked loss of color in 1, 1′, 2, 2′, 4, and 5, and the fluids in these tubes are now only pale pink. In 3, 3′ and 6 there has been only a moderate fading.

April 6.—About one-tenth of the color is left in 1 and 1′; i. e., 1 c. c. of the red fluid from a check tube diluted with 9 c. c. of water gives a color a trifle deeper than that in these tubes. Only one-twelith to one-fifteenth of the color remains in 2 and 2′. In 3 about one-fifth of the color remains, in 3′ about one-eighth, in 4 about one-tenth, in 5 about one-ninth, in 6 about one-seventh. In 7–10 there is no fading.

April 11.—The cultures still fall into three groups, i. e.: (a) Those in which nearly all of the color has disappeared, viz, *Ps. hyacinthi*, *Ps. campestris*, and *B. pyoc. pericarditidis*. (b) Those in which a considerable portion of the color remains, viz, *Ps. stewarti*, *B. coli*, and *B. amylovorus*. (c) Those in which the color remains the same deep red as on the start, viz, *Bacillus carotovorus*.

April 18.—About one-twentieth of the color is left in 1 and 1′; precipitate yellow. Only about one-fortieth of the color remains in 2 and 2′. In 3 there is about 5 times as much color as in 1 and 1′; in 3′ about twice as much. The color in the latter tube is Ridgway's rose pink. The precipitate in 3 and 3′ is yellow; it is most abundant in 3′. No. 4 is like 1 and 1′; precipitate white. In 5 and 6 the color is rose pink; precipitate white, more copious in 5 than in 6. In 7–10 a slight white precipitate and no change in color.

April 29.—Color gone in 1 and 1′. On looking through the fluid endwise there is a trace of vinaceous buff, but held up vertically to the light (16 mm. diameter) it appears colorless. Nos. 2, 2′, and 4 are like 1 and 1′, and there is no change in 7–10, i. e., it is as red as on the start. The rest of the tubes (3, 3′, 5, and 6) still show some color.

May 16.—Color has not entirely disappeared from 3, 3′, 5, and 6. The color in the 4 tubes of *B. carotovorus* is now only one-half as deep as it was on April 29. The rest are still colorless.

Litmus.

For tests made with litmus see under Reduction experiments and in various other parts of this paper.

REDUCTION EXPERIMENTS.

Methylene Blue.

The reducing tendencies of *Ps. hyacinthi* and other organisms were tested on methylene blue in Dunham's solution (1 per cent peptone and 0.5 per cent sodium chloride in distilled water). To each 100 c. c. of the Dunham's solution, which was made from Witte's peptonum siccum, was added 2 c. c. of a solution of 50 mg. of methylene blue in 50 c. c. of distilled water. The results obtained are expressed briefly in the following table, each organism grew in the medium, but as there was no repetition of the experiment some of the statements may be subject to revision:

Table VII.—*Effect of Ps. hyacinthi, etc., on methylene blue in salted peptone water. Experiment begun March 21. Color of fluid, bright blue.*

Species.	Reduction.	Effect of shaking.	Color at close of experiment.	Precipitate.	Duration of experiment.
Ps. hyacinthi (2 tubes).	Distinct (within a few days), and long continued. Mar. 27, about one-third and one-half as blue as checks. Apr. 11, one-thirtieth and one-fiftieth as much color as in checks. Apr. 18, still nearly reduced. Ps. hyacinthi has a marked effect on methylene blue in this solution, and for a long time. Apr. 25, color begins to return. May 2, as much, and nearly as much, color as in checks.	Color returns quickly and is blue.	Bright blue. Does not deepen on shaking.	Not stained	56 days.
Ps. campestris.....	Distinct (within few days). Mar. 30, one-half as blue as check. Apr. 11, one-fiftieth as blue as check.	Color deepens rapidly on shaking.	Green. Does not change on shaking.	.....do.....	Do.
Ps. stewarti	None. (Observations on Mar. 24, 27, 30; Apr. 6, 11, 18, 25; May 2, 16.) Feebly but distinctly clouded in March, then clear.	No change ...	Bright blue. Same color throughout progress of experiment as on start.	Deep blue .	Do.
B. pyocyaneus pericarditidis (1 tube).	Distinct (on Mar. 30). Apr. 6. Fluid uniformly pale greenish instead of bright blue; distinctly unlike Ps. hyacinthi and Ps. campestris; they are paler than this tube but rapidly deepen their color on shaking, whereas this does not change much even on prolonged shaking. Apr. 11, about one-fifth the color remains. Apr. 25, one-sixth as much color as in check tubes.	Does not change much even on long shaking. Fluid uniformly pale greenish.	Green. Does not change on shaking.	Not stained	Do.

TABLE VII.—*Effect of Ps. hyacinthi, etc., on methylene blue in salted peptone water. Experiment begun March 21. Color of fluid, bright blue*—Continued.

Species.	Reduction.	Effect of shaking.	Color at close of experiment.	Precipitate.	Duration of experiment.
B. coli (1 tube)	Doubtful. Soon heavily clouded. Paler blue at first (in March), then doubtful. No reduction. Possibly the paler blue on start was due to the heavy clouding.	Does not change. Uniformly blue.	Blue. Does not change on shaking.	Dark blue .	Do.
B. amylovorus (1 tube).	Doubtful. No distinct reduction. Less clouding than in B. coli.	Does not change.	Blue. Does not change on shaking.	Deep blue.	Do.

From the above table the 6 organisms mentioned appear to fall into 4 categories:

(1) Marked reduction, prompt reoxidation on shaking, final color the same as at the beginning—i. e., pure blue. Precipitate not stained. *Ps. hyacinthi.*

(2) As in 1, but the final color of the fluid green. *Ps. campestris.*

(3) Distinct slow destruction of color. Color does not return on shaking. Final color green. Precipitate unstained. *B. pyocyaneus pericarditidis.*

(4) Reduction feeble or doubtful or absent. Final color of the fluid blue. Bacterial precipitate stained deep blue. *Ps. stewarti*, *B. coli*, *B. amylovorus.*

INDIGO CARMINE.

The reducing tendencies of *Ps. hyacinthi* and other organisms on indigo carmine were tested in the same way as in case of methylene blue. The culture medium consisted of 100 c. c. of Dunham's solution, to which was added 2 c. c. of a solution of 500 milligrams of indigo carmine in 100 c. c. of distilled water. The results obtained are shown in the following table:

TABLE VIII.—*Effect of Ps. hyacinthi, etc., on indigo carmine in salted peptone water; tubes inoculated March 21; color blue.*

Organism.	Mar. 24.	Mar. 27.	Mar. 30.	Apr. 6.	Apr. 11.	Apr. 15.	Apr. 18.	Apr. 27.	May 16.
Check tube (uninoculated).			Color, pale Nile blue.	Greenish color has faded since last record.	Color gone			Colorless	
Ps. hyacinthi (2 tubes).	No distinct change in color.	Fluid a little bluer than in the check tube.	No reduction; fluid deeper blue than check tube.	Color, bright blue, i. e., in marked contrast to check tube.	Color, bright blue; precipitate yellow, 5 mm. broad.	Color, bright blue, but paler, i. e., fading.	Color slowly changing; it is now malachite green.	Color, pale chromium green, i. e., fading.	Color gone, except a pale dirty yellowish hue.
Ps. campestris	do	do	do	Not now pure blue, but a greenish blue, approximating Nile blue.	Color, paler than it was, i. e., a very pale green.	Color gone	Color gone	Color gone	Color gone.
Ps. stewarti	do	do	do	Not now blue; pale as check, i. e., a greenish color, decidedly paler than Nile blue.	Color gone		Color gone	Color gone	Color gone.
B. pyocyaneus pericarditidis.	do	do	do	Color still a bright blue; this tube showed the deepest blue on Mar. 30, i. e., pure blue.	Color, bright blue; precipitate 5 mm. wide.	Color, bright blue, but paler, i. e., fading.	Color, blue green; Ridgway's glaucous green.	Color nearly malachite green; deeper than tubes of Ps. hyacinthi.	A slight greenish hue still persists, i. e., an olive buff.
B. coli	do	do	do	Color not now blue, but a green as pale as the check.	Color gone		Color gone	Color gone	Color gone.
B. amylovorus	do	do	do	Not now blue; color a greenish blue, nearly as pale as check.	Color gone, i. e., no greenish blue remains.		Color gone	Color gone	Color gone.

As shown in this table, the development of a deeper, purer blue in all of the cultures, and the persistence of this color in *Ps. hyacinthi* and *B. pyocyaneus-pericarditidis* until long after it had disappeared from the check tube and from the other cultures, were the most interesting results obtained.

Litmus.

The action of *Ps. hyacinthi* on litmus (reduction, etc.) is shown in the following table:

Table IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars.*

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	242, litmus cauliflower broth.	.....	.....	5th day, no change.	9th day, no change in color.	27th day, no reddening on this date or earlier.	.....	14th day, begun; 18th, wholly reduced in bottom and paler in middle; 27th, complete.	No acid.
1	243, litmus potato broth.	.....	Good	.....	.....	18th day, no reddening of the litmus.	.....	18th day, becoming reduced in bottom.	Do.
1	373, milk with alcohol litmus.	Deep lavender; deep as mauve, but not same color; twice as deep as Ridgway's lavender.	.....	No record	10th day, slightly reddish in rim; 12th, more distinct reddish color in rim.	18th day, fluid retains its lavender color, but the rim has become rose color; 22d, fluid lavender, rim and pellicle reddish; 38th, rim and whole body of fluid pale reddish; 48th, curd pinkish lavender.	After 22d day and before 38th day.	? Not before 22d day; whey reduced on 38th.	Feebly acidified.
1	374, milk with alcohol litmus (twice as much as in 373).	A deep violet blue.	Good	.....do.....	10th day, rim bluer than in 2 checks; no other change; 13th, rim deeper blue, i. e., more indigo; no red.	16th day, as on 13th; 18th, rim begins to redden, body of fluid violet blue, a trifle bluer than checks; 22d, fluid deeper blue than checks, rim decidedly redder than it was, i. e., burnt carmine; 38th, rim red, fluid wine purple.	No separation (48 days).	No reduction ...	Do.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	393, milk with alcohol litmus.	Between mauve and aniline violet.	Good	No record	13th day, fluid redder, i. e., between lilac and rose purple; acid reaction appeared some days ago.	17th day, color near rose purple; 27th, rose purple; 50th, rose purple; 78th, rose purple.	No separation (78 days).	Slight on 13th day; none on 27th and 50th.	Germs alive on 50th day.
2	393, milk with alcohol litmus.	do	do	do	do	17th day, color near rose purple, 27th, rose purple; 50th, rose purple.	No separation (50 days); on slight simmering casein thrown down as an abundant flocculent precipitate.	do	Germs alive on 50th day; fewer alive in these two and the preceding than on 25th day; on boiling acid vapor and an agreeable smell suggestive of acetic acid; fluid acid after boiling for an hour.
3	394, milk with c. p. litmus.	Very dull hyacinth blue.	do	do	13th day, deeper blue than check.	17th day, deeper blue than check, no red reaction; 27th, deep blue, between hyacinth and cyanine; 50th, deep dark blue.	? The litmus itself precipitated some casein on steaming.	No reduction	No acid formed.
1	399, milk with c. p. litmus.	Deep hyacinth blue.	do	do	10th day, like checks; 14th, deeper blue than checks.	24th day, deeper blue than checks; 31st, deep blue; 48th, deep blue.	?	do	No acid.
1	399, milk with c. p. litmus.	do	do	do	10th day, like checks; 14th, deeper blue	24th day, deeper blue than checks no visible change in last 9 days; 48th, rim and	?	None on 24th or earlier; wholly reduced on	No acid formed from the wood alco-

					than checks; on 15th added 2 drops methyl alcohol.	top of fluid deep blue, remainder still reduced.		31st except bright blue patches on wall above fluid.	hol.
2	400, milk with c. p. litmus (more than in 399).	Deep blue	.....do	.....do	10th day, no change in color; 14th, deeper blue than checks.	24th day, deeper blue than checks; 31st, deep blue; 48th, deep blue.	?	No reduction ...	No acid.
1	430, milk with c. p. litmus.	Between Ridgway's lavender and royal purple.	Good; distinctly yellow pellicle from 4th day on.	3d day, no change; 6th, slight deepening of blue.	13th day, bluer than checks.	15th day, 13 mm. of loose sediment (flax-flower blue) has settled out of fluid; the latter is dull hyacinth blue; 50th, casein dull hyacinth blue, whey dark purplish; 65th, casein dark hyacinth purple, whey not red by reflected light.	After 9th day; on 13th 6 mm. bluish fluid over the blue casein; on shaking, the whole mass is fluid and uniformly blue and there is no immediate reseparation of whey (15 minutes).	None to 15th day; partial on 22d; the top 3 mm. casein gray, rest pale blue; 27th, only trace of color in whey (at top); casein very pale blue; 43d, wholly reduced.	No acid.
1	430 (litmus milk) with 2 drops methyl alcohol.	Between lavender and royal purple.	Good; 9th day, copious yellow surface growth.	3d day, no change; 6th, bluer than checks; 7th, bluer than checks.	9th day, a little deeper blue than checks; 13th, deeper blue than checks.	15th day, no reddening; 27th, casein dirty gray, whey dull hyacinth blue; 50th, casein deep hyacinth blue, whey dark purple; 65th, casein dull hyacinth blue, whey not red by reflected light.	After 9th day and before 13th.	None to 9th day; slight on 13th. Marked on 22d—i.e., more than in the preceding. Deeper part of casein still blue, upper part gray.	No acid.
1	430 with 4 drops methyl alcohol.	.....do	Good	2d day, no change; 5th, bluer than check.	8th day, deep blue—bluer than check, 10th and 11th, bluer than check, 12th, uniformly blue.	15th day, casein lavender blue, 12 mm. deep in a fine precipitate, whey hyacinth blue; 24th, whey dark hyacinth purple; 30th and 45th, casein brownish (still partly reduced); whey deep hyacinth blue.	10th day	24th day, casein dirty gray, 11 mm. deep.	No acid.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	430 with 2 drops ethyl alcohol (absolute).	Between lavender and royal purple.	Good	2d day, no change; 3d, no change; 5th, blue with some purple in it.	Reduced	24th day, color begins to return; the whey is pale burnt carmine; the casein is a dirty yellow gray; 45th, heated 10 minutes at 56° C.; 56th, casein purplish red, whey slightly red by reflected light.	5th day (probably on 4th).	7th day, partial; 8th, all reduced; 12th, all reduced; 30th, all reduced; 45th, all reduced.	Slightly acid.
1	430 with 4 drops ethyl alcohol (absolute).	.....do	Good; a yellow pellicle on 4th day.	4th day, fluid bluer than check; 5th, uniformly blue.	8th day, deep blue—bluer than check. No red color.	17th day, 24 mm. of deep lavender blue casein, 13 mm. dark colored whey; a wide yellow rim and copious yellow precipitate; 23d, whey becoming slowly red, even by reflected light (dull red); 38th, casein purplish gray, whey reddish; 49th, casein purplish red, whey pale red by reflected light.	8th day (1 mm. whey).	23d day, casein now gray.	Slightly acid.
2 tubes	441 (430+2½% grape sugar).	.....do	Good	1st, 2d, 3d days, no change; 4th, no reddening; 6th, no change in one tube; slight deepening of blue in other; 7th as on 6th.	9th day, color same as 2 checks; 13th, still uniformly blue and now deeper than the checks.	27th day, whey dull purple, casein pale lavender blue in one tube and deeper blue in the other; 50th, litmus now slowly oxidizing back; the whey is dark purple; upper part of casein dull blue in one tube and bright blue in the other; the deeper parts in each are gray (still reduced); 65th, casein blue, no purple in it, whey not red by reflected light.[1]	After 13th day, on the 15th, 2 to 3 mm. of whey on top of blue casein; 22d, casein has not solidified, but is slowly settling out as a blue finely divided precipitate. The visible whey is only 7 mm. deep.	After 13th day, partial on 22d and 27th; complete on 31st; casein gray; casein gray on 43d.	No acid reaction detected in either tube. Is peptone necessary?
1	442 (430+2½% cane sugar).	.....do	Good; excellent surface growth on 7th day.	1st, 2d, 3d days, no change; 6th and 7th, like check.	9th day, doubtful; if any change, not so blue; 13th, same color as	15th day, 9 mm. translucent whey on 45 mm. uniformly blue curd, which is a trifle paler than the check tube; 22d, whey nearly colorless,	After 9th day, on 13th, 1 mm. of whey on top of the uniformly blue	After 13th day, partial on 15th; 32d, casein pale blue; 43d,	Slight trace of acid at close.

					check. No red color.	casein blue, 3 cm. deep, not solidified, and settles very slowly; 27th, casein blue; 50th, color returning; whey pale brownish red, casein gray below, purple blue above; 65th, lower one-half of casein gray, upper one-half purple blue, whey pale purplish.	casein.	casein gray white, whey pale yellow; all reduced except a few pale blue flecks on yellow rim.	
1	443 (430+2½ galactose).	.....do.....	Good; excellent yellow surface growth on 7th.	1st, 2d, 3d days, no change; 6th and 7th, color like check.	9th day, no red; if any change in color, bluer; 13th, milk uniformly blue and slightly deeper than the check.	15th day, 3 to 4 mm. whey on top of the uniformly blue casein; color deeper than check; 22d, curd, uniformly blue, not solidified; about same color as check; as in all of the milk cultures the casein has separated out slowly as a voluminous, noncoherent precipitate; 27th, curd 3 cm. deep, blue, with a trace of purple in it; 50th, color oxidizing back; whey dull brownish red, upper one-half of casein deep hyacinth blue, lower one-half a dirty gray; 65th, casein hyacinth blue, whey dull wine red (reflected light).	After 13th day and before 15th.	After 13th day, partial before 22d (in whey); 27th, whey yellowish; 43d, whey pale yellow, curd grayish brown. No litmus color except in the yellow rim where there are many small pale blue patches.	Slight acidity in whey at close.
1	444 (430 + 1% maltose).	.....do.....	Good; copious surface growth on 9th day.	1st, 2d, and 3d days, no change; 6th, no change; 7th, a trifle lighter blue than check.	9th day, no red color, but a lighter blue than check; 13th, casein blue, but paler than check, not solidified.	15th day, slightly redder by reflected light than check tube; 22d, litmus in deeper parts of tube dull purplish blue; 27th, the litmus which is not reduced is pale purplish red by reflected light; 43d, fluid and casein dull purple; 50th, casein dull hyacinth blue; whey dull wine red (color is barely visible by reflected light); 65th, casein dark blue, no purple in it; whey dark by reflected light.	Very slight separation on 9th.	Doubtful, possibly some on 7th day; wholly reduced in upper 1 cm. on 22d; 27th, nearly reduced.	Slight acidity 3d and 4th weeks.

[1] In stocks 441 to 446 (exclusive of 2d maltose test) the organisms were all destroyed on the 43d day by heating them 15 minutes at 80° C., falling to 62° C.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	444 (430 + 1% maltose); inoculated a month later from another culture.	Between lavender and royal purple.	Prompt; good. On 17th day, a wide, bright yellow rim.	4th day, bluer; 5th, deep blue—i. e., bluer than when inoculated.	8th day, same as on 5th.	17th day, casein blue (R's lavender, but twice as deep a color); whey not red by reflected light; 23d, fluid dull purple by reflected light; 38th, heated 10 min. at 56° C.; 49th, casein bluer than it was (litmus reoxidizing), whey dark blue by reflected light.	7th or 8th day (1 mm. whey on 8th).	Doubtful: casein grey blue and whey yellowish on 38th day.	Very slight indication of acidity on 23d day.
1	445 (430 + 2½% mannit).	.....do.....	Good; distinct yellow pellicle on 4th day.	1st, 2d, and 3d days, no change; 6th and 7th, same color as check; no reddening.	9th day, no red color, doubtful if any change, possibly slightly bluer; 13th, fluid uniformly blue; slightly deeper than the check.	15th day, casein fills the greater part of the culture, and is a uniform opaque blue, deeper than the check; 22d, casein uniformly deep blue, and not coherent (solidified); it occupies all but upper 6 mm. of the culture: 27th, whey yellowish (reduced); most of culture occupied by the slowly settling casein, which is blue with a purplish tinge; 43d, fluid dull purple by reflected light; nearly all of the casein dissolved; 50th, casein dull hyacinth blue; whey dull wine red (reflected light); 65th, casein blue; whey dark (not red).	After 13th day and before the 15th, whey 4 mm. deep on 15th.	After 13th day. Partial in whey before 22d.	Slight indication of acidity on 27th, 43d, and 50th days.
1	446 (430 + 2½% c. p. glycerine).	.....do.....	A good surface growth on 7th day; 22d, bacterial rim bright yellow, but not over one-tenth as much as in other tubes.	1st, 2d, and 3d days, no change; 6th, like check; 7th, slightly bluer than check.	9th day, slightly bluer than check; 13th, milk uniformly blue, distinctly deeper than check.	15th day, bluer than check; 22d, distinctly bluer than check; 27th, fluid uniformly blue; growth slower than in the other tubes (430–445); 31st, a decided dulling of blue, but no acid reaction; 50th, color returning; fluid uniformly deep blue; 65th, fluid deep blue, wholly opaque; distinct yellow rim and precipitate.	No separation of whey from the casein.	After 15th day. Partial on 31st; nearly complete on 43d.	Growth retarded by the glycerine; no acid formed.

2	532 c. p. litmus (same formula as 430, i. e., 10 c. c. of a saturated water solution of dry, lime-free blue litmus to 200 c. c. of milk).	.....do.....	Good; a bright yellow pellicle 3d day.	2d day, purple rim above, cream has changed to blue; 3d, rim bluer; no change in body of milk; 6th, no red color, and no decided increase of blue; 7th, casein slightly bluer.	10th day, curd in one tube dark hyacinth blue, twice as deep as in checks; color in the other not quite so deep blue, but no indication of any acid; 13th, deeper blue than on 10th; one tube lags behind the other as if it had received fewer bacteria.	20th day, no indication of any acid reaction; litmus in the whey reduced, casein partly dissolved; unreduced litmus a dark plum purple.	Trace of whey (?) the 3d day; 4th, 2 mm. whey in one tube, 3 mm. in other; 7th, 7 mm. whey in one tube, 6 mm. in other.	Slight on 6th and 7th days, 10th, 11 mm. whey, of which four-fifths reduced in one tube and one-half in other; litmus in the casein not reduced.	No acid.

From an inspection of the foregoing table it is evident that, as a rule, under the conditions named, *Ps. hyacinthi* reduced litmus only very slowly. In litmus milk its first visible effect was a deeper bluing of the milk, which persisted for some time; the casein was then thrown down slowly, and a partial or complete reduction of the litmus usually followed. Upon reoxidation, the litmus was again blue. Addition of methyl alcohol led to no acid reaction. Addition of ethyl alcohol caused the development of a slight quantity of acid, which inhibited further growth, but did not immediately destroy the organisms. This acid is volatile and the boiling culture smells like acetic acid. Glycerin retarded growth, no acid was formed, and the casein did not separate. Addition of other substances to the litmus milk—e. g., mannit, galactose, cane sugar, grape sugar—led (during the first few weeks) either to the formation of no acid or to the production of so slight a quantity that it was easily obscured by the alkali.

Ps. campestris and *Ps. phaseoli* were also tested in litmus milk and other litmus cultures. In general, their behavior was like that of *Ps. hyacinthi*. The milk first became deeper blue, the casein was then thrown down slowly, and the litmus was reduced. In some cases, at least, the litmus was reduced more rapidly by these two organisms than by *Ps. hyacinthi*. On reoxidation the litmus was blue.

The relative rapidity of the reduction of litmus is worth noting. For instance, in some broths tinctured with this substance and inoculated with *Ps. hyacinthi*, all of the litmus color disappeared except in the uppermost layers in contact with the air, but this reduction took place slowly, requiring several weeks, where *Bacillus cloacæ* consumed only as many days.

In a litmus cauliflower broth inoculated with *Ps. hyacinthi* reduction was first visible toward the end of the second week and was not complete until after the third week. In the same broth inoculated with *Ps. phaseoli* reduction began to appear at the end of the first week and was complete at the end of the second week. In the same broth inoculated with an undescribed organism belonging to the *B. cloacæ* group[1] there was partial reduction of the litmus in 20 hours and complete reduction in 48 hours.

Ps. stewarti cultivated in the same litmus milk behaved differently. It grew well, but the casein was not thrown down and a slight amount of acid was formed. This is usually not observable the first week and it is often obscured for a long time by the reducing action of the organism. The action of this germ on litmus milk is shown in the following table:

[1] Isolated from rotting potato tubers received from Florida and designated in the writer's notes as "The Florida gas-forming wet rot."

TABLE X.—*Behavior of Ps. stewarti in litmus milk.*

	Stock.	Color.	Growth.	Color changes.			Separation of casein.	Reduction.
				First week.	Second week.	Third week and subsequently.		
Ps. stew ..	373	Very deep lavender; deep as R.'s mauve, but lavender color.	Good.		9th day, no fading of color, no reddening; 13th, no visible change.	128th day, milk now lilac color, opaque; slime on wall out of fluid is 2 cm. wide and reddish yellow, i. e., dragon's blood or madder, with a dash of yellow.	No separation (128 days).	Reduced between 13th and 128th.
Ps. stew ..	373	.....do	34th day, considerable yellow precipitate.	No change for a number of days.		34th day, no reddening of fluid to date; color has slowly faded to a very pale lavender, much paler than R.'s No. 16, i. e., almost white, a copious yellow rim which begins to be reddish; 153d, fluid lilac, opaque; rim on wall out of fluid 1 cm. wide, reddish yellow.	No separation (153 days).	Slow bleaching of litmus, not visible for some days.
Ps. stew ..	394	Very dull hyacinth blue.			13th day, fluid deep purplish blue; no purple in corresponding tubes of *Ps. hyacinthi*.	17th day, more purple and less blue than on the 13th; color distinct from check and suggestive of deep prune purple; 27th, fluid purple-red.	No separation.	?
Ps. stew ..	399	Between hyacinth blue and plum purple.			10th day, very slight change from check, as if a trifle of reddening; 14th, a distinct change, i. e., a slight admixture of rose; color lies between mauve and heliotrope purple.	24th day, milk is now a uniform heliotrope color; the checks have remained unchanged; 31st, changes are going on very slowly; the milk is now a uniform dull heliotrope; on the bottom under the buff yellow precipitate the litmus is once more blue over a few sq. mm.; 48th, a centimeter breadth of red slime on wall out of fluid.	No separation.	No reduction to 14th day; 48th, litmus reduced to a dirty gray-white, with a trace of pink in it.
Ps. stew ..	400				10th day, blue, but redder than check; change slight; 14th, heliotrope purple.	24th day, a dull purple, approximating Indian purple; 48th, wide purple rim (bacterial) on walls above fluid.	Casein separated by excess of litmus in preparation of media.	No reduction on 10th day; 48th mostly reduced.

TABLE X.—*Behavior of Ps. stewarti in litmus milk*—Continued.

	Stock.	Color.	Growth.	Color changes.			Separation of casein.	Reduction.
				First week.	Second week.	Third week and subsequently.		
Ps. stew ..	430		Good; on 13th a copious yellow precipitate rim and pellicle.	1st to 6th day, no change; 7th, slightly bluer than check.	9th day, deeper blue than the checks; 13th, deeper blue than checks.	15th day, no reddening; 22d, color now very unlike that of check; over one-half the color is discharged, the tint now being a uniform pale lavender; 27th, color pale lavender except in bottom, where it is whiter; 32d, color lilac; 43d, lilac; 50th, color lilac; 65th, color now lies between lilac and heliotrope purple.	No separation (65 days).	None to 13th day; slight at bottom on 15th; on 22d, uniform slow reduction.
Ps. stew ..	532			2d day, purple litmus above cream has become blue; no other change; 3d, increased bluing of rim; 6th, fluid paler, but still lavender blue; 7th, rim purple, milk lavender, but paler or redder(?) than checks.	10th day, milk has slowly changed color; it is now heliotrope purple; 13th, as on 10th.	20th day, on the wall above the yellow pellicle the litmus is purple; milk pale, but not white or gray; it is a color belonging to Ridgway's series on Plate VIII; it came nearest to a mixture of lavender and lilac or lavender and heliotrope purple; the purple of the rim is deeper, i. e., more like Indian purple.	No separation.	On 6th day litmus uniformly paler than in checks; 13th, more reduction (at top).

TESTS FOR HYDROGEN SULPHIDE.

The tests for H_2S were made by suspending in the tops of test tubes, containing cultures of *Ps. hyacinthi*, narrow strips of filter paper which had been dipped in a saturated water solution of c. p. lead acetate. The strip was held in place by having its upper end wedged between the wall of the tube and the close-fitting cotton plug. The following trials were made:

(1) *Coconut culture.*—Growth good. Paper introduced on fourteenth day. Result: Strip feebly browned in 24 hours. Removed and inserted another moister paper. In 48 hours the lower 1 cm. of the strip was distinctly brown, but not deep brown.

(2) *Coconut culture (from another series).*—Growth good. Paper introduced on fourteenth day. Result: Very marked browning of the lead acetate paper in 48 hours. After 3 weeks the strip was black in lower one-half inch, and brownish for another one-half inch. The bacterial layer was bright yellow and the substratum unstained.

(3) *Carrot culture.*—Growth good. Paper introduced on the ninth day. Strip examined and remoistened on fifth day. Result: No browning of the paper so long as the experiment continued (42 days). Substratum browned.

(4) *Potato culture.*—Growth good. Paper introduced on fourteenth day. Result: No browning of strip so long as under observation (3 weeks). Substratum grayed. Fluid feebly browned.

(5) *Rutabaga culture.*—Growth good. Paper introduced on third day. Result: No browning of the lead paper in 47 days. A slow browning of substratum, and bacterial slime.

(6) *White radish culture.*—Growth good. Paper introduced on third day. Result: No stain of the strip in 61 days. Substratum browned.

(7) *Yellow globe turnip culture.*—Growth good. Paper introduced on third day. Result: Seventh day, copious growth; no stain of the lead paper. Eighteenth day, a slight browning of the strip at bottom and a feeble browning of the upper part of the substratum. Twenty-seventh day, a feeble browning of the lower part of the lead paper; distinct pale browning of the upper part of the substratum. Thirty-fourth day, a slow increase of the brown color in the lead paper; slime neutral. Sixty-fourth day, only a slight browning of the lower end of the lead acetate paper; substratum brown (burnt umber); fluid grown full (solid) with yellow-brown slime; reaction acid.

Conclusion: *Ps. hyacinthi* caused prompt browning of lead paper when grown on sulphur-bearing substrata, which did not stain brown. With one exception, there was no evolution of hydrogen sulphide (browning of lead paper) when grown on substrata which became gray or brown as a result of the growth of the organism, although some of these must have been very rich in sulphur compounds. Query: Was the H_2S fixed in the substratum as fast as formed, by ammonia, with the resultant brown stain? See The Brown Pigment.

Ps. campestris behaved in much the same way. The lead paper was promptly browned when exposed over cultures on coconut, and the substratum was not stained. Exposed over potato and rutabaga, there was no browning of the paper, but a brown staining of the substratum. Exposed over white radish and yellow globe turnip, on

which growth was prompt and very copious, the paper browned slowly and the substratum also finally changed to brown. In a tube of radish there was no visible browning of the paper up to the fourteenth day of exposure, and on the same date there was only the merest trace of browning on the lower margin of the strip in the tube of yellow globe turnip. On this date there was an equally good growth in the 2 tubes, but there was no stain of the substratum in the tube of radish, while there was a distinct browning of the whole substratum in the tube of turnip.

Ps. stewarti grayed potato cylinders, but did not brown the lead paper (9 days' exposure). On rutabaga and yellow globe turnip it neither browned the paper nor stained the substratum (64 days). Also, on white radish in 64 days the substratum was not stained, but no test was made for H_2S.

Bacillus coli and an undetermined white organism (received as *B. coli* from the bacteriological laboratory of the Army Medical Museum), grayed potato cylinders promptly, but there was no browning of the lead acetate paper in 58 days.

For behavior of *Ps. phaseoli* see The Brown Pigment.

FORMATION OF INDOL.

The pink or red indol reaction was obtained with *Ps. hyacinthi* by adding sulphuric acid and sodium nitrite to cultures in Dunham's solution, in peptonized sugar-free beef broth, and in peptonized Uschinsky's solution. My practice was to add to the culture 15 drops of a mixture of sulphuric acid and water (2 acid, 1 water), and then 1 c. c. of distilled water containing 0.1 per cent sodium nitrite. If the color did not come at once, or within a few minutes (which was frequently the case), the tubes were plunged into water at 75° to 80° C. for 5 minutes, during which the color appeared. The color was a distinct red or pink. Uninoculated tubes tested at the same time gave no such reaction. Cultures of various ages were used, but none less than 3 weeks old. Old cultures must be used to obtain a distinct reaction, and in none was the color more than one-quarter as deep as that in corresponding tubes of *Bacillus coli*. In no case could any indol reaction be obtained from culture fluids which did not contain peptone. The same result was obtained with *B. coli* and a half dozen other organisms used for comparison. The presence in the culture fluid of peptone (using this term in the commercial sense) appears to be necessary for the production of indol.

The indol reaction was also obtained from cultures of *Ps. campestris*, *Ps. stewarti*, and *Bacillus amylovorus*.

TESTS FOR NITRITES.

PEPTONIZED BEEF BROTHS.

Two stocks were used: (1) A strongly alkaline beef broth (stock 382) with addition of 1 per cent Witte's peptonum siccum; (2) a slightly alkaline beef broth deprived of its muscle sugar by growing *B. coli* in it for 17 hours in the thermostat. This latter was clarified with the whites of 4 eggs, which were neutralized by HCl, and fortified with 2 per cent Witte's peptone. These cultures were tested on the twenty-second day after good growth. Neither gave any nitrite reaction with the indol-sulphuric acid test, the indol being that normally present in the cultures.

PEPTONIZED USCHINSKY'S SOLUTION.

This stock consisted of Uschinsky's solution with the addition of 1 per cent Witte's peptone. The tests were made at the end of 22 days. There was no nitrite reaction with the indol-sulphuric acid test, the indol being that normally present in the cultures.

NITRATE BOUILLON (STOCK 474).

This consisted of—

Distilled water, 1,000.0.
Witte's peptone, 10.0.
Beef extract, 2.5.
Chemically pure potassium nitrate, 3.0.

and sodium hydrate sufficient to render the fluid +10 of Fuller's scale.

Ps. hyacinthi grew readily in this medium without gas production. Examinations for nitrite were made on the sixth, sixteenth, and twentieth days, using the iodine-starch test—i. e., to each tube was added 1 c. c. of thin boiled starch water, 1 c. c. of one-half per cent potassium-iodide water (which should be freshly prepared), and finally a few drops of a fluid consisting of 2 parts of c. p. sulphuric acid and 1 part of distilled water. No trace of nitrite reaction could be obtained with this reagent. Subsequently grape sugar was added to some tubes of this nitrate bouillon (100 milligrams per 10 c. c.), but even in the presence of this agent *Ps. hyacinthi* was unable to reduce any nitrate to nitrite (8 days). Tubes of *Bacillus coli* and of *Bacillus carotovorus* were used for comparison. These became blue-black, like ink, on addition of the sulphuric acid.

Ps. campestris and *Ps. stewarti* resemble *Ps. hyacinthi*. Neither one is able to reduce potassium nitrate to nitrite in peptonized bouillon cultures, either with or without grape sugar. Comparisons were also made with *Bacillus amylovorus* and *B. pyocyaneus pericarditidis*. The former does not reduce nitrates to nitrites. The latter (like various

other green-fluorescent bacteria) first converts the nitrate to nitrite and then liberates the azote as free nitrogen.[1] Gas bubbles were given off continually during the first few days, so that the top of the fluid was foamy, as if it had been shaken violently. During this stage the liquid gave a deep blue-black reaction with boiled starch water, potassium iodide, and sulphuric acid. Later the gas bubbles disappeared and then (sixteenth day) no nitrite reaction could be obtained. The experiment with *B. pyo. pericarditidis* was repeated, using fermentation tubes; a considerable quantity of gas collected in the closed end. This gas was not absorbed on shaking with caustic soda (absence of CO_2), it did not diffuse quickly or explode when it was tilted into the open end of the tube and a lighted match applied (absence of hydrogen and marsh gas); lighted matches thrust into the bowl were repeatedly extinguished (presence of nitrogen).

One or two other interesting facts were observed in connection with cultures in the nitrate bouillon. *Ps. stewarti* and *B. amylovorus* made a very feeble growth in comparison with *Ps. hyacinthi* and *Ps. campestris*. *B. coli* grew better, throwing down in 16 days about 10 times as much precipitate as *B. amylovorus*. In early stages of growth, i. e. during the first 2 or 3 days, the + cultures of *Ps. campestris* were very different from those of *Ps. hyacinthi* in that the former contained many hundreds of tiny white zooglœæ scattered uniformly through the liquid, giving it, especially under the Zeiss × 6 aplanat, a distinctly granular appearance. On the sixteenth day this phenomena had disappeared and the cultures of the two organisms were then much alike.

Ps. phaseoli was also tested in this nitrate bouillon. Like *Ps. campestris*, it formed great numbers of small zooglœæ during the first few days of growth. It was entirely unable to reduce the nitrate to nitrite in this solution (14 days).

FERMENTS.

No attempt has been made to isolate any ferment, but the behavior of *Ps. hyacinthi* in the host plant and in various culture media leads to the conclusion that several enzymes are secreted.

Cytase.

The thin, non-lignified walls of the spiral vessels of the host plant are dissolved, letting the bacteria out of the vascular system into contact with the parenchyma. Fragments of the spiral threads are apparently all that remain of these vessels in bundles which have been long occupied. Once in contact with the parenchyma, cavities, filled by the bacteria, are formed in this tissue, the cells being first separated from each other and finally destroyed, as Dr. Wakker has described. These facts indicate the secretion of a cytohydrolytic enzyme. At the same time the slowness with which the vessels are

[1] These are the organisms that reduce the value of the farmer's manure pile.

destroyed and the cavities formed lead me to think that this substance is secreted only in extremely small quantities. The results of growth on different vegetable culture media point to the same conclusion. No softening of the cell walls was observed in any of the following substrata: Potato, sweet potato, sugar beet, coconut. A softening of the middle lamella of carrot, turnip, and radish cylinders was noted in old cultures.[1]

A few observations were made on the related organisms. Potato, coconut, rutabaga, yellow globe turnip, and radish cylinders were not softened by *Ps. stewarti*. *Ps. campestris* softened cylinders of potato, rutabaga, and yellow globe turnip.

The behavior of *Ps. campestris* in the interior of various host plants, in the absence of any other organism, indicates that a cytase must be present, i. e., closed cavities are formed. During the formation of these cavities, which are fully occupied by the bacteria, the parenchyma cells are first separated from each other by a multiplication of the organism in the intercellular spaces, the walls of the cells are then crushed together by the continued multiplication of the bacteria, and become more and more indistinct, until they finally disappear altogether.

In properly fixed, paraffin-embedded material, cut in serial section, all stages of the solution of the cells and the formation of these bacterial cavities may be readily observed, especially in the easily sectioned cabbage and turnip occupied by *Ps. campestris*. The organisms find their way into the parenchyma from the vessels, which are first occupied in ways already described by the writer elsewhere. That the destruction of the cell walls can be due to nothing but this organism, in the disease under consideration, is shown clearly as follows: (1) Because these are closed cavities, i. e., not in open connection with the surface of the plant, except at long distances from the place of occurrence; (2) because these cavities occur as freely in the interior of plants that have become diseased from the writer's pure-culture inoculations as they do in those which have become diseased naturally in the fields; (3) because the microscope shows the cavities to be filled exclusively by bacteria; (4) because cultures made from the interior of such inoculated and diseased plants have shown *Ps. campestris* to be the only organism present; (5) because all stages in the destruction of the cells and in the formation of these cavities can be followed in serial sections, so fixed and otherwise prepared that the relation of the bacteria to the various parts of the host plant is the same as in the living plant.

Ps. phaseoli also forms cavities in the interior of the host plants. Concerning *Ps. stewarti* I am in doubt.

---

[1] Since this was written, and too late to determine experimentally, it has occurred to me, as the result of reading Potter's papers, that possibly this solvent action on the middle lamella is due to the formation of *acid ammonium oxalate*. It cannot be due to oxalic acid as such since this has no solvent action on turnip tissues.

INVERTASE.

A slant tube of 10 per cent cane sugar agar, fragments of which gave no precipitate of copper oxide on boiling 2 minutes in Soxhlet's solution, gave after *Ps. hyacinthi* had been grown on it for 29 days, a very copious rusty precipitate after boiling 2 minutes in the same solution. Cane-sugar bouillon gave the same result. This indicates that cane sugar is inverted, and to a much greater extent than is needed for the growth of the organism, but we may not therefore assume the existence of an invertase. The fact that cane sugar was not inverted when put into dead or sterile tubes of *Ps. hyacinthi* cultivated in beef broth and peptonized beef broth, seems to show either that the living organism itself is necessary to bring about the inversion or else that invertase is formed only when it is required, i. e., in the presence of cane sugar.

My first experiments were in non-peptonized alkaline beef broth (stock 382). The contents of tubes 3, 4, 7, 10, 12, and 15 of February 7 (cover-glass inoculations 24 days old) were poured together and forced through a Chamberland filter. Two 10 c. c. portions of the sterile fluid were then pipetted into cotton-plugged sterile test tubes, and to each was added 300 milligrams of cane sugar. To one of these tubes chloroform was added and to the other thymol. They were then set away at 18° to 24° C.

On the fifth day each tube was tested by pipetting 2 c.c. of the clear fluid into boiling Soxhlet's solution, and continuing the boiling 1½ minutes. In neither case was there any reduction. These tests were repeated on the thirty-fifth day with the same negative result.

A duplicate series from tubes 1, 8, 14, and 18 of February 7 (same stock) led to the same result. In neither portion was there any reducing sugar on the fifth or thirty-fifth day.

Thinking that the invertase might possibly have been retained in the walls of the filter, or that the presence of peptone might be essential to the formation of invertase, the experiment was repeated as follows:

Three old cultures of *Ps. hyacinthi*—(1) in beef broth with Wittes's peptone (459); (2) in beef broth without peptone (382), and (3) in beef broth with the trace of muscle sugar removed by *B. coli* (404)—were sterilized by heating them for 10 minutes at 54° C., viz, at a temperature high enough to kill the organism and low enough to be harmless to invertase. To each tube was then added 500 milligrams of cane sugar and 150 milligrams of thymol. The sugar was transferred from a sterile solution by means of a sterile pipette. Along with these three cultures two other old cultures were tested, viz, one of *Ps. campestris* and one of *Ps. stewarti*, each in stock 382. These tubes were set away for 19 days at 25° to 30° C.

At the end of this period they were tested as follows for the presence of reducing sugars:

Twenty-five cubic centimeters of Soxhlet's standard alkaline solution was added to 25 c. c. of his standard copper sulphate solution, and after mixing was divided into 5 equal parts in 5 clean porcelain capsules and 40 c. c. of distilled water added to

each one. The fluid in one of these capsules was then brought to a boil and 1 c. c. from one of the tubes was added to it and the boiling continued for 1½ minutes. In the same way each of the other tubes was tested. In none of the 5 capsules was there any reduction of the copper.

A more conclusive test would be to grow these organisms in sugar bouillon for some weeks and then determine per cubic centimeter the exact copper-reducing power of the cultures. These should then be heated 10 minutes at 54° C., or thereabouts—i. e., long enough to destroy the organisms. Thereupon, measured volumes should be pipetted into sterile cane-sugar solutions. To similar solutions should be added equal portions from the cultures after heating them for 10 minutes at 80° or 90° C.—i. e., long enough to destroy the supposed invertase. Then after some weeks, if the fluids have remained sterile, their reducing powers should be determined quantitatively. An experiment of this sort was begun with *Ps. hyacinthi*, but was lost through a contamination which was probably introduced with the thymol. At least the intruding white organisms were capable of growing in the presence of an abundance of this antiseptic at a constant temperature of 50° to 52° C.

As the writer has had no opportunity to repeat the experiment, the question of an invertase must be left an open one. This only is tolerably certain—none is formed in the absence of cane sugar.

All of these 4 yellow organisms invert cane sugar readily, as already pointed out.

Diastase (Amylase).

The experiments with starchy media, already described, show that the diastasic action of *Ps. hyacinthi* is very feeble. Nevertheless, some growth occurred, even when the greatest care was taken to exclude all carbohydrate food except pure starch; and as tests with iodine water and with Soxhlet's solution showed that there had been a slight action on the starch, minute quantities of a diastatic ferment must be secreted. The starch which has been acted upon gives the red or amylodextrine reaction with iodine. *Ps. stewarti* acts on starch slowly, after the manner of *Ps. hyacinthi*.

On the contrary, *Ps. campestris* and *Ps. phaseoli* destroy starch and amylodextrine promptly in considerable quantities, so that in course of a few weeks none, or very little, is left in the culture tube, even when there were several grams of starch at the outset.

Experiments with both *Ps. campestris* and *Ps. phaseoli* showed that starch was converted in the absence of the bacteria (tubes heated for some minutes at a few degrees above the thermal death point and some of the fluid then added to potato starch with antiseptic precautions) and that none was converted if before adding them to the starch the fluids were heated to a point above that at which diastase is destroyed.

Trypsin.

A peptonizing ferment must be present, since gelatin and Lœffler's solidified blood serum are liquefied, and casein is slowly dissolved with the formation of tyrosin. This, also, is secreted in minute quantities or else is partially inhibited by other substances, because gelatin is liquefied very slowly even under favorable conditions—i. e., optimum temperature, proper alkalinity, and suitable food.

Ps. campestris and *Ps. phaseoli* behave in the same way—i. e., they liquefy gelatin and Lœffler's solidified blood serum and dissolve (peptonize) casein. These processes take place more rapidly than in case of *Ps. hyacinthi*, but in none of them is the peptonization speedy. *Ps. stewarti* does not liquefy gelatin or Lœffler's solidified blood serum.

Lab Ferment.

The existence of a lab or rennet ferment is at once suggested by the fact that in milk cultures the casein is thrown out of solution in the absence of any visible production of acids (see Milk and litmus milk and Litmus under reduction experiments). The casein is also precipitated if the whey from old alkaline milk cultures is first sterilized by heating it for ten minutes at 56° C. and then added to tubes of sterilized milk along with thymol. Media inoculated from the thus coagulated milk remained sterile.

The same whey, after heating for ten minutes at 90° C., had no effect upon milk.

Ps. campestris and *Ps. phaseoli* behave in the same way. Both throw down casein by means of a lab ferment. *Ps. stewarti*, on the contrary, produces no lab ferment and never coagulates milk.

Oxidizing Enzymes.

No oxidase or peroxidase was detected—i. e., the cultures of *Ps. hyacinthi* did not react blue with sensitive guaiac resin in alcohol nor was there any bluing on the subsequent addition of hydrogen peroxide. *Ps. campestris* behaved in the same way. The brown stain is believed to be due to other causes.

A copious evolution of gas bubbles took place when hydrogen peroxide was added to old potato cultures of *Ps. hyacinthi*, *Ps. campestris*, *Ps. phaseoli* and *Ps. stewarti*.

Such copious evolution of oxygen is, however, not peculiar to these particular parasites. It has been more recently observed by the writer in case of old potato cultures of *Bacillus coli*, *B. amylovorus*, *B. pyocyaneus pericarditidis*, a fluorescent germ obtained from fermenting tobacco and able to grow in the presence of thymol, Earle's bacillus of tomato fruit rot, an orange colored clumpy organism from cotton

leaves, a dendritic yeast, and a nondendritic yeast (both yeasts were obtained from the sticky surface of Niagara grapes). In all of these cases the gas soon foamed over the top of the test tube. An old coconut culture of *Ps. hyacinthi* also gave a very considerable quantity of gas.

The least amount of gas was obtained from adding H_2O_2 to 3-months-old potato cultures of Jones' carrot rot bacillus. Three tubes were tried, all of which behaved alike. At first there was no gas, then a slow, long continued evolution of small bubbles, the total not being very great. A young potato culture of this bacillus (8 days old) yielded gas almost from the start and in much greater quantity than the old cultures. The reverse was true of *Ps. campestris*. A potato culture 3 months old yielded gas more promptly and in greater volume than did a vigorous culture made from the same tube and only 8 days old. Both, however, yielded much gas. On the contrary, even that from the young cultures of Jones's bacillus fell far behind in amount that which was evolved by the other 10 bacteria and by the two yeasts.

Some gas was also obtained by pouring H_2O_2 upon old rice cultures of various fungi, e. g., *Fusarium niveum*, *F. vasinfectum*, Swingle's Atta fungus (cultivated by the writer from a nest of Atta, near Washington), and from an agar plate culture of cotton anthracnose.

The yield of gas from the fungi named was insignificant in comparison with that obtained from the yeasts and from the bacteria, exclusive of the old cultures of Jones's bacillus. The other bacilli and the two yeasts gave so much gas that the tubes were filled and frothed over, usually within a few minutes.

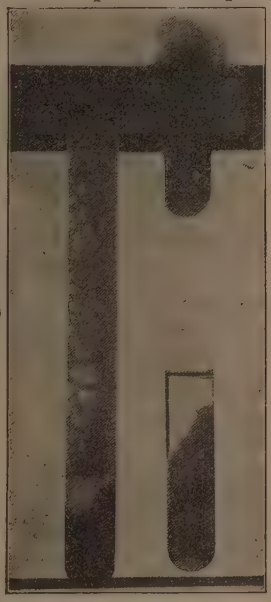


FIG. 1. (a) Evolution of gas on adding hydrogen peroxide to potato culture of *Ps. phaseoli;* (b) Uninoculated tube, to which hydrogen peroxide has just been added.

In the accompanying illustration (fig. 2) a 3-months-old potato culture of *Ps. phaseoli* to which H_2O_2 has been added is shown by the side of a check tube (uninoculated) to which H_2O_2 has also been added. In the one case there was a very copious evolution of gas bubbles, which filled the tube; in the other there was only a very slight evolution of gas, which soon ceased.

On heating a 3-months-old culture of *Ps. phaseoli* for 25 minutes at 75° to 85° C., and then adding the H_2O_2, there was no evolution of gas

at first, but after a few minutes bubbles began to be given off and a small amount of froth collected, but not over one five-hundredth as much as from the unheated tube. This tube was, of course, full of a thick yellow slime, which perhaps conducted heat badly.

In a second test, a similar potato culture of *Ps. phaseoli* was exposed for 2 hours at 85° C. On then adding H_2O_2 there was no evolution of gas either immediately or after a time. A similar potato culture of *Ps. campestris*, treated in the same way, behaved the same—there was no evolution of gas. The above illustration would serve equally well for the behavior of tubes of *Ps. campestris* or *Ps. phaseoli* before and after heating to 85° C.

As already shown, both *Ps. phaseoli* and *Ps. campestris* when grown on potato produce an abundance of diastase, but the breaking up of the H_2O_2 with liberation of oxygen can hardly be due to that enzyme, for a potato culture of *Ps. hyacinthi* of the same age as the preceding gave an enormous quantity of gas, although, as usual, it had made a rather meager growth (owing to its feeble diastasic action). This potato gave a strong starch reaction with iodine potassium iodide. Stearns & Co.'s pancreatic diastase also failed to cause any evolution of gas when it was dissolved in water and H_2O_2 added.

Dr. Oscar Loew has given reasons for believing that this decomposition of hydrogen peroxide is due to a hitherto unsuspected oxidizing enzyme, which he has named *catalase*,[1] and which he believes to be universally distributed in plants and animals and to have to do with respiration.

PIGMENT STUDIES.

The Yellow Color.

Dr. Wakker appears to have been uncertain whether the yellow color was inherent in the organism itself or only in a gummy substance surrounding it.

The yellow color of *Ps. hyacinthi* can not be shaken loose or filtered away from the bacterial cells by water, and, with the exception of nutrient starch jelly containing glycerine, it was never imparted to any of my fluid or solid culture media, whether neutral, acid, or alkaline. It pertains only to the bacteria themselves. Working in a good light with the best appliances at my disposal, viz, Zeiss 2 mm. apochromatic, 1.40 n. ap., with 12 and 18 compensating oculars, it has never been possible to locate the yellow pigment in any gum or granules lying between the cells. In my opinion the color is lodged *within*

[1] (1) Physiological Studies of Connecticut Leaf Tobacco. Department of Agriculture, Washington, D. C., 1900; (2) Catalase, a new enzyme of general occurrence, with special reference to the tobacco plant. Report No. 68, United States Department of Agriculture, Washington, Government Printing Office, 1901, pp. 47.

the organism, and is insoluble in water because it is dissolved in an oil secreted by the cells. The small size of the rods and the minute quantity of pigment in each one, has made it impossible to decide whether the color is lodged in the cell wall or in the cytoplasm itself. In whichever place, it appears to be uniformly distributed.

The intensity of the color depends, of course, on the density of the growth and also to some extent on its age and on the nature of the culture medium. It is always a distinct yellow. In the host plant it is chrome yellow to pale cadmium. It is also bright yellow on many culture media, especially when grown in the dark, e. g., gamboge, chrome yellow, or canary yellow. Occasionally, in cultures, it has been as pale yellow as primrose or maize yellow, but this has been the exception, and in some of these very pale cultures many involution forms were present. On some media, but not on all, old cultures became brownish or dirty yellow. In such cultures the slime has been dull yellow, dirty yellow, dark yellow, brownish yellow, ochraceous, and between ocher yellow and tawny olive. In young cultures, and in old cultures in which the brown stain was not detected the following shades of yellow have been seen: Primrose, maize, Naples yellow, wax yellow, gallstone yellow, saffron yellow, buff yellow, Indian yellow, gamboge, chrome yellow, deep chrome, lemon yellow, and canary yellow. The color was very dull in potato broths, but the whitish rim from such tubes made a homogeneous bright yellow growth when rubbed on suitable culture media. The color was also dull yellow in acid (unneutralized) beef broths, but in alkaline (soda) ones of the same origin it was bright (canary) yellow. The color was bright yellow in strongly alkaline gelatin and also in cane-sugar gelatin which had been acidified with malic acid. Excess of malic acid in the gelatin appeared to favor the development of the color, it being decidedly brighter in +54 than in +36 gelatin. The color did not appear to be any brighter when the organism was grown in the ice chest at 8° to 12° C. than when grown (in an equally dark place) at room temperatures of 25° to 30° C.

This color appears to be an oxidation product. It forms abundantly only in organisms near the surface of solid and fluid cultures. It is bleached by reducing agents, and regains its color after these have been removed. It does not form so abundantly when the organism is grown on suitable media in air containing a considerable reduction of free oxygen, i. e., on potato or coconut in nitrogen or carbon dioxide mixed with air. In these gases, when pure, there is no growth. In partial vacuum growth is less abundant and the color is paler yellow than in air. The same is true in nitrogen containing some oxygen, i. e., in air with the oxygen incompletely removed. (See Aerobism.)

The following substances bleach this color: Sulphuric ether, chloroform, turpentine, benzine, benzole, xylol, toluol, carbon bisulphide

(contaminated with H_2S),[1] and nascent hydrogen. The loss of color was most rapid in the carbon bisulphide, 30 to 60 minutes sufficing, in some cases, to render the bright yellow bacterial slime as white as white lead. On removing this fluid, which was neutral to litmus, but the vapor from which browned lead acetate paper, the yellow color began to return in a few hours and finally became nearly as bright as before. The other substances reduced the color more slowly, and on their removal it was a much longer time in coming back, and never became quite as bright as at first. The test with hydrogen was made as follows: The pigment was extracted by 23 days' exposure to c. p. glycerin. Into this yellow glycerin was then thrown a small scrap of zinc and some 30 per cent c. p. HCl., which caused a continual evolution of gas. On the sixth day the yellow color was still visible; on the seventh day it was nearly gone; on the tenth day it was all gone. On the thirteenth day the zinc was removed from the now colorless fluid. The fluid remained colorless for some days (a week or two). It then very gradually changed to yellow; 54 days after the removal of the zinc it was still only feebly yellow. The yellow color was not dissolved out by any of these reducing substances; at least no yellow stain was imparted to the fluids. The bacteria were hardened by alcohol, ether, and chloroform into tough masses not easily divided. Similar masses remained soft under xylol, toluol, and turpentine, and had an unctuous feeling when touched with a glass rod. Owing to the hardening action of chloroform, the writer uses it in preference to turpentine or xylol in passing the tissues from absolute alcohol into paraffin. In sections cut therefrom the tissues of the host plant will tear or become displaced more readily than the bacterial sheet.

This pigment is slowly soluble in glycerol, as Wakker pointed out. It is also soluble in water containing hydrogen peroxide, in ethyl and methyl alcohol, in acetic ether, and in acetone. The latter proved the most ready and satisfactory solvent, most of the color being removed in 30 to 60 minutes. Ethyl and methyl alcohol and ethyl acetate are rather slow and feeble solvents. The color is slowly soluble, without destruction, in strong ammonia water; it is quite soluble in water saturated with ammonium carbonate. It is also soluble on long standing in glacial acetic acid. It is insoluble in petroleum ether. It was not dissolved or changed by remaining 30 days in $\frac{N}{10}$ HCl. The color was not destroyed by steaming 25 minutes in water, nor by boiling in strong ammonia water. It was not reduced by steaming in water containing grape sugar.

The acetone extract appears to be sensitive to light. On exposing

[1] This is the carbon bisulphide which was used in my experiments with *Ps. campestris* (Centralb. f. Bakt., 2 Abt., Bd. III, page 479).

40 c. c. of the yellow acetone extract for some hours to bright sunshine on a tin roof, at 50° to 60° C., the fluid was reduced to 1 c. c., but instead of being an intense yellow, as was expected, it became a very pale yellow—i. e., there was less yellow in the 1 c. c. remaining than in the same quantity of the original fluid.

This color is not an oil, but seems to be intimately associated with such a body. On evaporating a yellow acetone extract (organism grown on sugar beet) to one-tenth or one-twentieth of its volume, the perfectly clear fluid became whitish cloudy, like an emulsion, and on examining it under the microscope it was seen to be composed of innumerable round bodies having the optical and chemical properties (osmic acid test) of oil globules. The yellow color was visible where these globules were massed, and also in noncrystalline patches, but separate oil globules did not appear to be yellow.

On driving off the remainder of the acetone with gentle heat, a small amount of chrome yellow, oily looking and oily feeling fluid remained in drops on the bottom of the white capsule. On adding concentrated c. p. H_2SO_4 to these yellow wet-shining drops there was an immediate decided blue-green reaction, which quickly changed to brown and soon after to brown-purple. After one-half hour an oily looking rim of brown-purple granules surrounded the drops of acid. This purple color was also fugitive, fading to a dirty gray. The acetone which was used changed to a clear brown on adding c. p. H_2SO_4, but with no preliminary blue-green color. The yellow residue which remained on evaporating the acetone extract from another lot of cultures (organism grown on coconut) changed at once, on adding concentrated c. p. H_2SO_4, into a green, which soon faded to purple. On adding the acid directly to the bacterial slime dried on glass slides it became orange-brown, then rusty-brown, and finally rose-brown, but no green or blue color appeared.

The presence of highly organized nitrogenous bodies is not necessary to the formation of this color. It is produced readily in Uschinsky's solution, with starch substituted for glycerin, and on this medium the yellow color is as pure and as bright as it is in the host plant or on coconut, sugar beet, peptone agar, or sugar gelatin.

These results lead me to think that this yellow color belongs to the Lipochrome group of plant pigments. (See Zopf: Die Pilze, p. 144.)

So far as I have tested it, the yellow pigment of *Ps. campestris* behaves in the same way, i. e., it is soluble in glycerin, ethyl alcohol, methyl alcohol, acetone, ammonium carbonate in water, and glacial acetic acid; it is insoluble in sulphuric ether, chloroform, xylol, toluol, or carbon bisulphide, but is bleached by these substances. As a rule, the yellow color of *Ps. hyacinthi* is brighter than that of *Ps. campestris* or *Ps. phaseoli*.

THE BROWN PIGMENT.

Under certain circumstances, not clearly understood, a pale brown pigment, soluble in water, is also produced by *Ps. hyacinthi*.

This feeble brown stain occurs in the host plant; in hyacinth broth; in alkaline peptonized beef broth (after 5 or 6 weeks); in one-half strength potato broth with 1 per cent Witte's peptonum siccum (not when the peptone is omitted); in the same peptone potato broth with addition of malic acid; on radishes (49 days, not in 25 days), white turnips, and yellow turnips; on banana pulp and banana rinds; and in water surrounding potato cylinders, the potato itself being grayed.

This pigment did not appear in any of the following media, not even in very old cultures: Acid beef broths (55, 59, 75, 80 days); alkaline beef broths free from peptone (33, 67, 71, 97, 100, 119 days); alkaline beef broth with cane sugar (26, 39, 67, 82, 98 days); distilled water with 4 per cent maltose, 4 per cent dextrine, and 4 per cent Witte's peptone (29, 40 days. Doubtful at the end of 70 days—no brown stain in one tube and a slight (?) browning in the other); 4 per cent peptone water (15 days); one-half strength potato broth (73 days); the same with small amounts of caustic soda (59, 73 days); Uschinsky's solution (83 days); standard agar containing some muscle sugar, acidity +22 of Fuller's scale (22 days); standard agar containing no muscle sugar, acidity +15.5 (13, 18, 47 days); the preceding agar with grape sugar (18, 29, 47 days); the same with cane sugar (29, 47 days); the same with fructose (31 days); litmus alkaline gelatin (39 days); malic acid gelatin (34 days); malic acid gelatin with cane sugar (174 days); gelatin neutral to phenolphthalein with soda (87 days); the same with cane sugar (61 days); sugar beet (55, 60, 67, 70 days); coconut (49 days, 95 days); potato with cane sugar (2 tubes, 67 days—a third tube showed slight browning on sixty-seventh day, but less than tubes without the sugar); nutrient starch jelly made from Uschinsky's solution by substituting washed potato starch for the glycerol (35, 62 days); the same with Taka diastase (39, 62 days); the same with maltose (30 days); the same with dextrine (30 days).

In the inoculated hyacinth plants the brown stain was not very noticeable, being confined, so far as observed, to the vascular bundles of the diseased leaves, and easily overlooked. In nutrient media the pigment does not appear immediately and is best observed in old cultures (1 to 3 months). It is never as pronounced, either in the host plant or on culture media, as the similar pigment formed by *Ps. campestris*. The most decided browning was in old cultures on cruciferous substrata and on banana skins. My failure to obtain any browning in gelatin cultures contradicts Dr. Wakker's statements, but this contradiction may be apparent rather than real—i. e., dependent, possibly, on differences in the chemical composition of the nutrient gela-

tins employed. On the other hand, it is probable that the browning he observed arose from the presence of some intruding organism—e. g., the one which produced gas bubbles in his gelatin.

The following shades of brown were observed: (1) A slight browning (yellow banana pulp, 55 days; water around potato cylinders, 31 days); (2) feeble brown (white turnip, 22 days; water around potato cylinders, 24, 37, 67 days; washed potato starch cooked in distilled water with 4 per cent Witte's peptone, 73 days; hyacinth broth, 59 days); (3) pale brown (yellow turnips, 23 days; one-half strength potato broth with 1 per cent Witte's peptone, 41, 59, 73 days; the same with malic acid, 41, 73 days); (4) brownish (a potato cylinder with 500 mgs. Merck's diastase of malt absolute, 41 days); (5) feeble reddish brown and later brownish white with the slightest trace of pink (washed potato starch with 4 per cent peptone water and Taka diastase, 44, 73 days; also the same stain without the diastase but feebler); (6) tawny olive (white turnips, 40 days); (7) paler than tawny olive (yellow turnips, 22 days); (8) ochraceous (white radish, 50 days); (9) russet (white turnips, 40 days); (10) between russet and burnt umber (yellow turnips, 40 days); (11) light burnt umber (white turnips, 49 days); (12) midway between burnt umber and mummy brown (yellow globe turnip, 50 days); (13) burnt umber (yellow globe turnip, 64 days); (14) sienna with a very slight admixture of brown (radish, 49 days); (15) dark brown (skin of yellow banana, 56 days).

The formation of this pigment appears to depend on the presence of certain highly organized nitrogenous bodies—e. g., albuminoids or peptones. Whether it is produced inside of the bacterial cell and dissolved out, or is formed in the substratum by the chemical action of colorless substances excreted from the cells, as seems more likely, could not be determined. Its formation also appears to depend on the presence of free oxygen, as in one instance, in an old culture on rutabaga (50 days) it was observed that the upper part of the substratum was distinctly browned, but in that part protected from the air (the lower one-half of the cylinder, in water grown full of the yellow slime and solidified) it was not browned.

Ps. stewarti grayed potato cylinders, but in two months it formed no brown pigment in tubes of radish, rutabaga, or yellow globe turnip. In from 6 weeks to 2 months *Ps. campestris* stained these same cruciferous substrata various shades of brown—e. g., (1) raw sienna, (2) a color between russet and cinnamon rufous, (3) a color between russet and tawny olive, (4) raw umber, (5) burnt umber, (6) dark burnt umber, (7) mummy brown.

These brown pigments are also believed to be in some way connected with the presence of sulphur compounds and of tannins or related bodies in the plant or substratum, and with the formation of hydrogen sulphide and ammonia by the bacterial organism.

As we have already seen, H_2S is given off promptly from coconut cultures of *Ps. hyacinthi* and *Ps. campestris*, which do not become grayed or browned, and is not given off from potato or carrot cultures, which do become stained.

A graying of steamed potato cylinders with subsequent pale browning of the water in which they stand—viz, a change similar to that induced by many different sorts of bacteria—is readily produced by adding to the tubes a few drops of ammonium sulphide. Tannin, in the air, is oxidized readily to deep brown compounds when exposed to ammonia, but this change does not take place in vacuo neither did the potato cultures gray in vacuo. All bacteria or nearly all produce ammonia and hydrogen sulphide, and many vegetable substances contain tannins or allied compounds.

A somewhat different result was obtained with *Ps. phaseoli*, which also grays potato cylinders and becomes dulled in color by the formation of a small amount of soluble brown pigment. My attention was drawn especially to this by the behavior of some potato cultures. Eight of these were alike in their yellow color and the substratum was grayed; the ninth, while alike in all other cultural respects, was a much brighter yellow, and there was no distinct stain of the potato. At the time I had in the laboratory two stocks of potato made from different tubers. The question now arose whether the bright chrome yellow culture was specifically different from the wax yellow cultures, or had been made accidentally on the newer potato stock and was the same species, but different in color on account of some slight chemical difference in the culture medium. To test this latter hypothesis a tube from each potato stock was now inoculated from the bright yellow culture. In one of these daughter tubes the growth was dull wax yellow, and the substratum was distinctly grayed within 48 hours. In the other the equally abundant growth was bright chrome yellow, exactly like the culture from which it was made. There can be no doubt, I think, that the usual dulling of the slime of *Ps. phaseoli* on potato is to be ascribed to the absorption of a brown pigment formed out of some substance commonly present in the substratum. These two cultures made from the bright yellow culture were tested for H_2S on the fifth day by placing strips of lead acetate paper in the top of the tubes under the cotton plug. The paper in the dull yellow culture browned promptly. That in the bright yellow culture did not brown at all at first (24 hours), but finally browned feebly, corresponding to a slowly appearing feeble gray color in the substratum. When the cultures were 9 days old and the paper had been exposed 4 days the conditions were as follows: The tubes were alike in volume of growth and in general appearance, except as given below. In one the color was a dull wax yellow, the lead paper was dark brown at the lower end, the substratum was distinctly grayed,

and the bacterial slime reacted immediately and distinctly alkaline to neutral litmus paper. In the other tube the color was bright yellow (gamboge), the lead paper was feebly browned (only about one-tenth as much as in the preceding), the substratum was very feebly grayed, and the bacterial slime reacted differently to the neutral litmus paper—i. e., it was exactly neutral.

Ps. phaseoli cultivated on yellow and white turnips made a good growth, but no brown pigment was observed. On turnip-rooted radishes the growth was also good and there was no brown stain for a month, but after that a slight stain appeared.

NATURE OF THE CELL WALL.

The bacterial cell wall of *Ps. hyacinthi* stains yellow with iodine potassium iodide, and remains yellow on the addition of sulphuric acid (Russow's cellulose test). Tests were made with germs grown on agar, potato, starch jelly, etc.

The bacterial slime from cultures on banana and sweet potato reacts blue with Russow's test. At first this was supposed to indicate cellulose in the bacterial wall. Subsequently it was discovered that the blue reaction is due to some substance which may be washed away in water, the bacterial masses then giving only a yellow stain. This substance, which reacts blue, is believed to form no part of the bacterial cell, but to be the dissolved substances of the substratum, carried up and held between the bacterial cells by capillarity. These experiments were repeated a year later with banana, using old cultures which bore a thick, dull yellow slime. This slime gave no blue reaction with iodine potassium iodide (absence of starch), but a very decided blue on adding sulphuric acid. Several washings in water greatly reduced the tendency to this blue reaction, but did not entirely prevent it. This was believed to be due to the fact that the water had not penetrated into the center of all the bacterial masses. The experiment was therefore repeated as follows: Masses of the surface slime aggregating 30 or 40 cubic millimeters, entirely free from fragments of the substratum (which contained undestroyed starch), were shaken in a beaker with 150 c. c. of distilled water, and then put under an air pump for one-half hour, so as to remove air from the slime and permit penetration of the water into all parts. This water was then poured off, more added, and the exhaustion repeated. This second water was also poured off, more added, and the beaker again put under the air pump. After the third exhaustion there remained several hundred small bacterial fragments (zoogloeæ). As much as possible of the water standing over them was then poured off and 4 c. c. of iodine potassium iodide was poured into the beaker and allowed to act for 20 minutes, during which time all of the fragments became yellow. To this fluid was then added 4 c. c. of the c. p. sulphuric acid water (2:1). In an

hour's time there was not the faintest trace of any blue reaction, all of the bacterial fragments remaining yellowish brown. Some unwashed masses of bacteria, carefully removed from the surface slime of one of these banana cultures, were now thrown into the beaker. Their surface immediately blued, and in a few minutes each one of these masses became deep blue throughout, forming a very striking contrast to the yellow stain in the washed particles.

It may be that substances absorbed from the substratum into the bacterial layer will account for all of the few recorded cases of cellulose reaction in the bacteria. This possible source of error is certainly worthy of very careful consideration.

VITALITY.

Length of Life in Culture Media.

No special attention was given to this subject, but from time to time, for various purposes, tubes of suitable culture media were inoculated from old cultures. The results show that *Ps. hyacinthi* is not readily destroyed by its own decomposition products. The nature and age of the old cultures in which this organism was still alive are given below: Feebly (litmus) alkaline potato broth, 24 days; beef broth neutral to phenolphthalein, with 5 per cent cane sugar, 32 days; moderately alkaline beef broth, with 10 per cent cane sugar, 79 days; acid (unneutralized) beef broth, 26 and 64 days; feebly (litmus) alkaline slant agar, 24 days; nutrient starch jelly, 31 days; sugar beet, 52 days; coconut, 59 days; white turnip, 41 and 80 days; radish, 80 days; gelatin neutral to phenolphthalein, 38 days; gelatin alkaline to phenolphthalein, i. e., −20 of Fuller's scale, 156 days; malic acid gelatin (acidity +54 of Fuller's scale), with 10 per cent cane sugar, at temperatures ranging from 10° to 25° C., 174 days. In a potato culture 91 days old the organism was dead. It was also dead after 33 days in a beef broth made feebly alkaline to litmus by sodium carbonate. It was dead in 5 coconut cultures at the end of 2 years; it was dead on sugar beet after 2 years and 10 months; it was dead in 3 cultures on agar (stock 527) after 17½ months. These results indicate that the organism is fairly resistant, and also that it produces very little organic acid.

Ps. campestris, *Bacillus amylovorus*, *B. carotovorus*, *B. pyocyaneus pericarditidis*, and a green fluorescent germ which grows in the presence of thymol, and which was isolated by the writer from one of Dr. Loew's tobacco infusions, were all alive on agar (stock 527) after 17½ months. *Ps. stewarti*, on the contrary, was dead (2 tubes). *Ps. phaseoli* was also dead. All of these tubes were in the stock-culture box, subject to the same degree of cold (temperature 5° to 16° C.), moisture, and darkness. Under similar conditions *Ps. campestris* was

alive on potato after 5 months and on agar (stock 553) after 10 months. Several tubes of *Ps. phaseoli* were alive after 5 months on potato. *B. carotovorus* was also alive on potato at the end of 5 months.

Resistance to Dry Air.

Dr. Wakker states that the hyacinth organism remains alive in a dry state for a long time. Only three experiments were made to determine this point, all of which tend to confirm his statement.

(1) A typical potato culture 9 days old was shaken until nearly all of the yellow slime was washed into the 1 c. c. of fluid in the bottom of the tube. Fifteen small drops of this heavily clouded fluid was then spread on 15 small, clean, sterile cover glasses, in a Petri dish, the cover replaced, and the dish set away in a dry, dark closet, at 20° C., for 9 days. At the end of this time 13 of these covers were dropped into as many tubes of culture media—beef broth, sugared peptone water, etc.

Result: *Ps. hyacinthi* developed after a few days in all of these tubes, showing that some germs were still alive on each cover glass. The time required to cloud these tubes was 3 to 8 days, at 16° to 20° C.

(2) The remaining 2 covers were kept until the 47th day, after which they were put into 1 per cent grape sugar peptone water.

Result: After a few days the fluid in each tube clouded and threw down a yellow precipitate.

(3) Some weeks later this experiment was duplicated, with the exception that a period of 48 days was allowed to intervene between the spreading of the cloudy fluid on the covers and their submersion in the culture medium. In this instance the bacteria were derived from a 9-days-old culture on yellow banana, the slime being rubbed over the clean sterile covers, which were then set away as before. On the 48th day 18 of these covers were seized with sterile forceps and dropped into as many tubes of sterile beef broth (stock 382) and set away in the dark, at 20° to 26° C.

Result: All but one of these tubes developed *Ps. hyacinthi*. Nine clouded on the 4th day; 5 on the 8th day; 2 on the 12th day. Two tubes were clear on the 17th day, but one of these was cloudy on the 23d day. The other remained clear. These results seem to indicate a marked difference in the vitality of individual rods. These are the cultures which were tested for invertase.

Experiments with *Ps. campestris* and *Ps. phaseoli* show that they are also resistant to dry air, but apparently less so than *Ps. hyacinthi*. The organisms were dried on cover glasses in a dark closet in the same way as *Ps. hyacinthi*, except that the temperature averaged about 5° higher. The covers were inoculated copiously and were side by side in clean covered Petri dishes. Of course those inoculated from the

potato received the most bacteria. The tests were made from solid and fluid cultures and into two kinds of beef bouillon, viz: (1) Stock 577, a standard salted peptonized beef broth +15 of Fuller's scale, which had dried out one-fourth by long standing; (2) stock 579, a flask of stock 577 diluted with an equal bulk of distilled water before it was filled into the test tubes. When everything was ready for the test, the dishes were brought out of the closet, and in clean, still air the inoculated covers were seized with sterile forceps and dropped one after another into the tubes of beef bouillon, which were then replugged, set away in the dark, and watched for six weeks.

These experiments were as follows:

(1) *Ps. campestris.*—Covers inoculated copiously from the yellow slime on a potato culture 2 days old. Dry 34 days.

a. Covers thrown into stock 577—12 tubes.

Result: One tube clouded on the 3d day and one on the 4th day. The other 10 remained clear. The clouding was typical for this organism, and cultures made from each of the tubes into potato yielded a typical growth of *Ps. campestris.*

b. Covers thrown into stock 579—12 tubes.

Result: Six tubes were cloudy on the 3d day; 6 remained clear. The clouding was typical, and cultures from each of the 6 tubes into tubes of potato yielded in each case pure cultures of *Ps. campestris.*

(2) *Ps. campestris.*—Each cover inoculated with a small drop of moderately cloudy fluid from a beef broth culture 2 days old. Dry 34 days.

a. Covers thrown into stock 577—11 tubes.

Result: No growth in any of the tubes.

b. Covers thrown into stock 579—12 tubes.

Result: Two tubes clouded on the 3d day. The rest remained clear. The clouding was typical, and transfers from the tubes into tubes of potato yielded pure cultures of *Ps. campestris.*

(3) *Ps. phaseoli.*—Covers inoculated copiously with the yellow slime from a potato culture 3 days old. Dry 27 days.

a. Covers thrown into stock 577—9 tubes.

Result: One tube clouded on the 3d day, one on the 5th day, and one on the 6th day. The rest remained free. The clouding was typical, and cultures from each tube into potato yielded a pure growth of *Ps. phaseoli.*

b. Covers thrown into stock 579—12 tubes.

Result: Two tubes clouded on the 3d day and 2 on the 4th day. The rest remained free. The clouding was typical, and cultures from each of the tubes into tubes of potato yielded a typical growth of *Ps. phaseoli.*

(4) *Ps. phaseoli.*—Each cover inoculated with a small drop from a well-clouded beef-broth culture 3 days old. Dry 27 days.

a. Covers thrown into stock 577—12 tubes.
Result: All clear to the end of the experiment.
b. Covers thrown into stock 579—10 tubes.
Result: No clouding in any of the tubes.

Conclusion: In each case the transfers from potato did better than those from beef broth. The dilute bouillon appeared to be a more favorable medium than the concentrated. *Ps. phaseoli* seems to be less resistant to dry air than *Ps. campestris.*

Resistance to Sunlight.

The writer's experiments have not been very numerous, and the shortness of exposure absolutely necessary for the destruction of the organisms is not known, but probably it is considerably less than the time given below. The tests were made in poured plates of nutrient agar, which was inoculated copiously. The exposures were made in very thin-bottomed Petri dishes lying bottom up on larger Petri dishes filled with pounded ice. The exposures were made in Washington in May. One-half of each plate was covered by several folds of thick paper and the other half exposed to the unclouded sun. A good-sized drop of well-clouded fluid was used in making each inoculation, i. e., many thousands of the bacteria. The bacteria in the covered portion of the dishes developed normally (except near the margin of the paper covering) as a dense uniform sheet of crowded small colonies. On the exposed part of each plate, and for some millimeters beyond, nearly all of the bacteria were destroyed. The few that remained were tardy in development and undoubtedly owed their escape to the protecting shade of less fortunate individuals. The exposed plates were as follows:

1. *Ps. hyacinthi.*—30 minutes' exposure; all killed.
2. *Ps. hyacinthi.*—45 minutes' exposure; all killed.
3. *Ps. campestris.*—30 minutes' exposure; 95 per cent killed.
4. *Ps. campestris.*—45 minutes' exposure; 98 per cent killed.
5. *Ps. phaseoli.*—30 minutes' exposure; 98 per cent killed.
6. *Ps. phaseoli.*—45 minutes' exposure; all killed.

The exposure was at midday. The temperature of the plates during the experiment ranged from 24° to 27° C., i. e., was held down satisfactorily by the ice. In each case a considerable portion of the bacteria were killed under that part of the cover nearest to the exposed portion, i. e., over a width of one-fourth inch or more. On this part the colonies developed slowly at first, and, being fewer, had more room to grow, and became larger than on any other portion of the covered part of the plates. The covered part of each dish was turned south, i. e., toward the sun.

Stewart found that exposure of *Ps. stewarti* in a portion of an agar

plate to bright sunlight for 3 hours destroyed nearly all of the organisms. In that part of the plate which was not exposed the yellow colonies came up thickly in 96 hours at 23° C.[1] He does not speak of having tried the result of shorter exposures. Russell and Harding found that exposure of *Ps. campestris* in agar plates for 15 minutes to an August sun (latitude 43°) destroyed 90 per cent of all the organisms. Similar cultures exposed for 30 minutes to a November sun remained entirely sterile.[2]

Resistance to Heat.

Ps. hyacinthi is quite sensitive to heat, much more so than the bacterial parasites of the warm-blooded animals. To a less degree the same is true of *Ps. phaseoli* and *Ps. campestris*. See Temperature relations.

Resistance to Acids.

Ps. hyacinthi is quite sensitive to acids, being restrained from growth by comparatively small doses. It will tolerate more acid in a solid than in a fluid medium, and more of some acids than of others. See Malic acid gelatin and Sensitiveness to acids. In beef broth with malic acid +30 appears to be about its limit of growth.

Resistance to Alkali.

Ps. hyacinthi will grow in −25 gelatin and in −20 beef broth, but experiments have not been numerous enough to determine just how much alkali it will endure. In gelatin and beef bouillon −30 of Fuller's scale is probably about the limit of toleration of caustic soda.

Growth in Presence of Calcium Sulphite.

Ps. campestris grew in 10 c. c. portions of galactose-peptone water with addition of 40 milligrams of calcium sulphite, but growth was distinctly retarded. The stock consisted of distilled water, ½ per cent peptone, and ½ per cent galactose. Other organisms were not tested.

Growth over Chloroform.

This test was made by adding 5 c. c. portions of c. p. chloroform to 10 c. c. portions of sterile alkaline beef broth in cotton-plugged test tubes. The beef broth was stock 382,[3] well adapted to the growth of this organism. The chloroform settled at once to the bottom, but

[1] A Bacterial Disease of Sweet Corn. Bulletin 130. N. Y. Ag. Exp. Sta., Geneva, N. Y., 1897, p. 434.

[2] A Bacterial Rot of Cabbage and Allied Plants. Bulletin 65. Ag. Exp. Sta., Wisconsin. Madison, Wis., 1898, p. 19.

[3] 1,320 grams minced lean beef and 2,000 c. c. distilled water in ice chest 24 hours. Steamed, filtered, resteamed, added water to make fluid 2,640 c. c. Titrated and found +25. Added caustic soda to 0. A fermentation tube yielded 0.6 c. c. gas with *B. coli*. No peptone added.

on unplugging its odor was always perceptible in the mouth of the tube.

The chloroform exerted a marked retarding influence on *Ps. hyacinthi*, but did not always prevent its growth. The tube was first inoculated with two 3 mm. loops from a 10-days-old moderately cloudy culture in Dunham's solution. In 24 days (at 20° to 25° C.) there was no growth. The tube was now reinoculated with two 3 mm. loops of cloudy broth from a 3-days-old culture in stock 382. After 12 days there was a faint surface clouding and a feeble partial rim of germs, which indicated that growth was proceeding with much difficulty. A month later there was a good, dense, yellow rim, 2 mm. wide, the fluid was well clouded, and on top of the chloroform there was a loose yellow bacterial precipitate about 2 mm. deep.

For comparison with *Ps. hyacinthi* tubes of the same medium were inoculated with other organisms. Under the same conditions *Ps. campestris* refused to grow. The tube was first inoculated with two 3 mm. loops from a 10-days-old moderately cloudy culture in Dunham's solution. After 24 days, there being no growth, the broth was reinoculated with two 3 mm. loops from a well-clouded 3-days-old culture in stock 382. After 43 days, there being no growth, the tube was inoculated for the third time with a 2 mm. loop of solid slime from a 48-hour growth on the surface of cooked turnip. This slime was broken up in the fluid by means of the platinum loop, and afterwards divided to a still greater extent by shaking the fluid thoroughly. The tube was under observation for an additional 13 days, but no growth ensued.

Ps. stewarti, on the contrary, grew in this chloroformed beef broth abundantly, with only slight retardation, and remained alive in it for more than 2 months. The tube was inoculated with one loop from a 10-days-old culture in Uschinsky's solution.

A number of other organisms behaved in much the same way as *Ps. stewarti*, i. e., they were more or less retarded for a few days, but afterwards made a more or less copious growth. Among these were *B. amylovorus*, *B. carotovorus*, *B. pyocyaneus pericarditidis*, and *B. coli*. *Ps. phaseoli* grew slowly in chloroformed cane sugar bouillon when inoculated copiously.

MEANS OF DISTINGUISHING THE FOUR SPECIES OF PSEUDOMONAS.

The four species of Pseudomonas may be distinguished as follows:

Found in .....
1. Cruciferous plants—cabbage, cauliflower, kohlrabi, kale, rape, turnips, rutabagas, mustards. *Ps. campestris.*
2. Leguminous plants—beans of various kinds, e. g., lima beans, bush beans. *Ps. phaseoli.*
3. Liliaceous plants—hyacinths. *Ps. hyacinthi.*
4. Gramineous plants—maize, especially sweet corn. *Ps. stewarti.*

Growth on steamed potato cylinders standing in distilled water.	1. Copious and prolonged, covering the potato and filling the water with a solid yellow slime and changing all of the starch within a few weeks so that it does not react blue or red with alcohol iodine or iodine potassium iodide. *Ps. campestris, Ps. phaseoli.* 2. Moderate and very little after the second week, not always covering all of the exposed part of the potato and never filling the water with a solid yellow slime, the starch but little acted upon and always yielding (even immediately under the slime) a pronounced blue, blue purple, or red purple reaction. *Ps. hyacinthi, Ps. stewarti.*
Growth in milk	1. The whey is slowly separated from the casein by means of a lab ferment; the casein slowly settles and after some weeks is partially redissolved. *Ps. campestris, Ps. phaseoli, Ps. hyacinthi.* 2. Growth good, but milk continues opaque and the whey never separates from the casein. *Ps. stewarti.*
Growth in litmus milk.	1. Blue litmus becomes gradually more and more alkaline. At no time is there any indication of acids. *Ps. campestris, Ps. phaseoli, Ps. hyacinthi.* 2. Blue litmus in course of some weeks changes to lilac or heliotrope, indicating the formation of a slight amount of acid. *Ps. stewarti.*
Growth on nutrient gelatin and Loeffler's blood serum.	1. A slow liquefaction, best in the order named. *Ps. phaseoli, Ps. campestris, Ps. hyacinthi.* The latter brightest yellow. 2. A good buff-yellow growth, but no liquefaction. *Ps. stewarti.*
Growth on steamed yellow turnips or rutabagas standing in distilled water.	1. Copious in the air and filling the fluid with a thick yellow slime, which is not iridescent; substratum browned and softened. *Ps. campestris, Ps. hyacinthi.* The latter Naples yellow, the former paler yellow. 2. Buff yellow, slightly iridescent, sparing (thin), and soon at an end, never filling the water with a solid yellow slime. Substratum not browned or softened. *Ps. stewarti.*
Growth in milk or bouillon with ethyl alcohol.	1. On boiling old cultures the steam yields an acid reaction and a fragrant smell. *Ps. hyacinthi.* 2. No such acid reaction or odor. *Ps. campestris, Ps. phaseoli, Ps. stewarti.*
Behavior in tomato juice and cabbage juice.	1. Did not grow. *Ps. campestris, Ps. phaseoli, Ps. hyacinthi.* 2. Grew copiously and for a long time without retardation (cabbage) or with only a slight retardation. *Ps. stewarti.*
Behavior in concentrated beef broth (acidity, +80).	1. No growth. *Ps. campestris, Ps. phaseoli, Ps. hyacinthi.* 2. Retardation for some days, then a copious and prolonged growth. *Ps. stewarti.*
Behavior in Dunham's solution with indigo carmine.	1. No immediate reduction; color slowly changes to a pure bright blue, which persists for several weeks, but finally fades through green to yellowish. *Ps. hyacinthi.* 2. No immediate reduction; color bluer for a few days only, changing to green and bleaching much sooner than the preceding. *Ps. campestris, Ps. stewarti.*
Behavior in Dunham's solution with methylene blue.	1. Marked reduction; on shaking, a prompt reoxidation (to blue); final color the same as at the beginning (pure blue); bacterial precipitate not stained. *Ps. hyacinthi.* 2. As above, but the final color of the fluid green. *Ps. campestris.* 3. No reduction, final color of the fluid blue; bacterial precipitate stained deep blue. *Ps. stewarti.*

Behavior in Dunham's solution with alcoholic solution of rosolic acid and a small amount of HCl.	1. Between the 6th and 9th day the pale orange yellow fluid changes to a geranium red, which gradually deepens to poppy red (37th to 56th day). *Ps. campestris.* 2. The color changes follow the same general course as in the preceding, but much more slowly; i. e., no distinct change of color until after the 16th day and not so deep on the 56th day. *Ps. stewarti.* 3. The yellow color of the fluid gradually bleached and practically all gone at the end of the second week; no reddening of the fluid. *Ps. hyacinthi.*
Behavior in chloroformed beef broth.	1. No growth. *Ps. campestris.* (Only one experiment.) 2. Slow, long-continued growth, but with much difficulty in getting started. *Ps. hyacinthi, Ps. phaseoli.* 3. Good growth, with little difficulty in getting started. *Ps. stewarti.*
Behavior in distilled water containing 4 per cent maltose and 4 per cent Witte's peptonum siccum.	1. Fluid in old cultures (40 to 60 days) distinctly browned. *Ps. campestris.* 2. Fluid not browned. *Ps. phaseoli.*
Growth on 10 c. c. slant nutrient agar containing 3 grams of cane sugar.	1. No distinct retardation, surface smooth, slime copious, and generally wet enough to flow readily. *Ps. campestris, Ps. phaseoli.* 2. Marked retardation of growth, surface roughened, reticulated or areolated, slime dry so that it does not flow. *Ps. hyacinthi.*
Behavior on 10 c. c. slant nutrient agar containing 1 gram of grape sugar.	1. Growth copious, stimulated from the start. *Ps. campestris, Ps. phaseoli, Ps. stewarti.* 2. Growth retarded for a week or more, but finally better than in the check tubes. *Ps. hyacinthi.*
Behavior on 10 c. c. of slant nutrient starch jelly containing 500 mgs. of glycerin.	1. Growth, after 24 days, copious, sirupy, bright yellow. *Ps. campestris.* 2. Growth, after 24 days, much less than in the preceding or than in the check tubes, and with no distinct yellow color. *Ps. phaseoli.*
Behavior on slant nutrient starch jelly made with modified Uchinsky's solution (see p. 63).	1. Growth good, slime yellow, marked diastasic action. *Ps. campestris.* 2. Growth much less abundant than in the preceding and slime very pale, marked diastasic action. *Ps. phaseoli.* 3. Growth feeble, no diastasic action. *Ps. hyacinthi.*
Behavior in Uchinsky's solution.	1. No growth or growth long delayed and feeble, with appearance in the fluid of small, whitish, loose, wooly flocks. *Ps. hyacinthi.* 2. Growth retarded and feeble, zoogloeæ compact, roundish. *Ps. campestris.* 3. As in 2, but a yellower and rather better growth. *Ps. phaseoli.* 4. An abundant and long-continued growth—a very suitable culture medium. *Ps. stewarti.*
Thermal death point (10 min. exposure in beef bouillon).	(1) 53° C. *Ps. stewarti.* (2) 51.5° C. *Ps. campestris.* (3) 49.5° C. *Ps. phaseoli.* (4) 47.5° C. *Ps. hyacinthi.*

Brightest color:
1. Generally wax yellow. *Ps. campestris.*
2. Wax yellow to chrome. *Ps. phaseoli.*
3. Chrome yellow to canary. *Ps. hyacinthi.* The brightest yellow of the four.
4. Buff yellow to chrome. *Ps. stewarti.*

N. B.—Old cultures darken and stress must not be laid on slight differences in color at any age, since the yellow color of the same species varies according to the amount of brown pigment produced, and this varies with the medium and sometimes even with slight changes in the medium (see page 142).

REMARKS ON THE YELLOW PSEUDOMONAS GROUP.

Characters in Common.

These bacteria agree in the following particulars: They are yellow rod-shaped organisms of medium size, straight or slightly crooked, with rounded ends. The segments multiply by fission after elongation. They are generally less than 1 μ in diameter. The segments are of variable length. As taken from the plant or from ordinary culture media, they are seldom more than three times as long as broad, and are often much shorter. The segments occur singly, in pairs or fours joined end to end, or in clumpy masses of variable size (zooglœæ), more rarely they are united into long chains or into filaments in which no septa are visible. Endospores are absent or rare (none have been observed). The segments are motile by means of one polar flagellum, which is generally several times as long as the rod, and may be wavy or straight when stained. The species grow readily on all of the ordinary culture media, but so far as definitely known all require the presence of air—i. e., are strictly aerobic.[1] None are gas producers. All are sensitive to sunlight. All are quite resistant to dry air. They do not reduce nitrates to nitrites. As a rule, they are not easily destroyed by their own decomposition products. The yellow color appears to be a lipochrome. In the different species it varies from deep orange and buff-yellow, through pure chrome and canary-yellow, to primrose yellow and paler tints. In the same species the yellow color also varies somewhat, being frequently changed, darkened, or obscured by the production of a soluble brown pigment, the amount of which pigment varies in different species, and in the same species on different media. Organisms parasitic in plants or saprophytic.

As our knowledge increases it will, of course, be necessary to revise this characterization and probably to subdivide the group. *Ps. campestris* and *Ps. phaseoli* are nearly related; *Ps. hyacinthi* differs from the above very considerably, and *Ps. stewarti* is still further removed.

[1] Note possible exceptions mentioned on pages 66, 67, and 71.

OTHER SPECIES BELONGING TO THIS GROUP.

The following species also belong to this group and appear to be distinct from the foregoing, but our knowledge of their cultural characters is more or less imperfect:

(1) *Ps. juglandis* Pierce. Parasitic on the young nuts, leaves, and stems of *Juglans regia* in California. The cause of an economically serious disease in walnuts. Resembles *Ps. campestris*. Pierce does not mention having attempted to inoculate his organism into cruciferous plants, but the writer has tried the reverse of this without success, viz, inoculations of *Ps. campestris* and *Ps. phaseoli* into young rapidly growing shoots of the walnut (*J. regia*).

(2) *Ps. vascularum* (Cobb). Parasitic on sugar cane in Australia and elsewhere. The vascular bundles are filled with a yellow slime, the canes are dwarfed, and the sugar content is reduced.

(3) *Ps. dianthi* (Arthur and Bolley). Isolated from carnations (Dianthus spp.), and supposed to be the cause of a spot disease. Common on the surface of carnation leaves, but now believed to be purely saprophytic.

(4) *Ps. amaranti* n. sp. Occurs on species of Amarantus (weeds in fields) in the Eastern United States, filling and browning the vascular bundles and hollowing out the tissues in their vicinity into closed cavities filled with this organism. The plants which are attacked are stunted, droop, and dry up without any visible cause. The organism is a short rod and when grown on culture media has more orange in its pigment than any others here described. On the whole, it seems to be most nearly related to *Ps. stewarti*.

(5) *Ps. malvacearum* n. sp. Parasitic on cotton (Gossypium spp.). This organism causes the very characteristic leaf disease known as Atkinson's angular leaf-spot, and also a water-soaked spreading spot-disease of the capsules comparable to that produced on walnuts by *Ps. juglandis* and on bean pods by *Ps. phaseoli*. This bacterium has nearly the same thermal death point as *Ps. campestris* and much resembles it in many other ways, but its slime is more translucent on potato, and it is not parasitic to cabbage. The writer has had this organism under observation for several years, and has successfully inoculated it into young cotton bolls and leaves. Tissues of the cotton plant which are not growing rapidly do not readily contract the disease. This yellow organism is not the same as the green fluorescent germ isolated by Stedman from rotting cotton capsules and named *Bacillus gossypina*. A full account of the cotton disease is in preparation.

21788—No. 28—01——11

O